The Journal of
Clinical Endocrinology and Metabolism

VOLUME 21 MAY, 1961 NUMBER 5
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AN XXXY SEX CHROMOSOME COMPLEX IN KLINEFELTER SUBJECTS WITH DUPLICATED SEX CHROMATIN

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ABSTRACT

Chromosome studies were carried out on 2 mentally defective male patients with Klinefelter's syndrome, whose interphase nuclei contained, in many instances, 2 masses of sex chromatin. In both patients the chromosome number was 48, or 2 in excess of the normal diploid number for man, in cells derived from blood, bone marrow and skin. Analysis of metaphase plates showed a chromosome complement of 22 pairs of normal autosomes and an XXXY sex chromosome complex. In one of the patients, cells derived from 1 of 4 skin biopsy specimens exhibited further chromosome anomalies that presumably arose during cultivation of the cells *in vitro*. Sex chromosome complexes XXY, XXXY and XXYY have now been demonstrated in chromatin-positive males with seminiferous tubule dysgenesis. The basic requirement in the cytogenetics

Received November 16, 1960.

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§ Operated by Union Carbide Corporation for the U. S. Atomic Energy Commission.

of the syndrome appears to be the presence of at least two X chromosomes in combination with at least one Y chromosome.

A PROPORTION of males with clinical manifestations of the Klinefelter syndrome (seminiferous tubule dysgenesis) have chromatin-positive nuclei (1–4) and in these patients the sex chromosome complex is XXY (5, 6). Barr *et al.* (7) described 2 mentally defective patients with the Klinefelter syndrome, whose nuclei in buccal smears and skin biopsy specimens were not only chromatin-positive but, in about 25 per cent of cells, contained 2 masses of sex chromatin. This unusual sex chromatin pattern suggested that the sex chromosome complex was even more anomalous than XXY. Ferguson-Smith, Johnston and Handmaker (8), using the bone marrow technic, found XXXY complexes in 2 males with duplicated sex chromatin in a proportion of nuclei. The present report, based on an independent investigation, confirms their observation in 2 additional subjects, using preparations derived from leukocytes of peripheral blood, bone marrow (1 patient) and skin.

PATIENTS

Mental retardation is one of the variable components of the Klinefelter syndrome (9, 10). Consequently, the frequency of the syndrome appears to be significantly higher in institutionalized mentally defective patients, among whom just under 1 per cent of the males have nuclei that are chromatin-positive, in comparison with the general male population (11–14). The 2 subjects whose karyotypes are to be described were mentally defective and were inmates of the Ontario Hospital School, Orillia.

Case 1

This patient was Case 1 in the report of Barr *et al.* (7); the earlier paper may be consulted for clinical details and illustrations. In summary, he was 15 years old, had an intelligence quotient of about 60, and a normal habitus for an adolescent male, except for small testes. Nuclei in a buccal smear and a skin biopsy specimen were chromatin-positive, about 30 per cent of them showing duplicated sex chromatin, and there were 6 neutrophils with drumstick nuclear appendages in 307 neutrophil leukocytes. Urinary excretion of gonadotropins was elevated and the testicular histopathologic picture was typical of that found in patients with the chromatin-positive Klinefelter syndrome.

Certain special studies and a number of laboratory tests were performed during the present investigation in view of the unusual karyotype that was demonstrated. Roentgenograms of the chest and skull, an electrocardiogram, and an electroencephalogram were within normal limits. The fasting blood sugar level was 80 mg. per 100 ml. and the glucose tolerance curve after oral administration of 100 Gm. of glucose was as follows: half an hour, 90 mg.; one hour, 85 mg.; one and a half hours, 63 mg.; and two hours, 61 mg. per 100 ml. The test was repeated, administering 100 Gm. of glucose by intravenous drip over a period of one hour in order to determine whether the failure of glucose levels to increase appreciably was caused by a slow rate of absorption. The blood sugar levels were then as follows: fasting, 79 mg.; half an hour after termination of the

glucose infusion, 318 mg.; one hour, 51 mg.; one and a half hours, 59 mg.; two hours, 75 mg.; and four hours, 76 mg. per 100 ml. The results point to an accentuated insulin response to the administration of glucose. The results of other laboratory tests are recorded in Table 1.

Case 2

This patient was a 14-year-old boy who was referred to the London group by Dr. A. Finlayson, Ontario Hospital School, Orillia, with a provisional diagnosis of the Klinefelter syndrome.

The boy's father was of dull normal intelligence and his mother was a behavioral problem. He was an only child, born when his mother was 20, and his father 23 years old. The parents were not consanguineous and birth conditions were normal. Mental retardation was not suspected until the child started school at the age of 5 years.

The report of Mr. F. A. Horton, Psychologist, Ontario Hospital, London, reads as follows:

"Intellectual assessment: The Wechsler Intelligence Scale for Children was administered, yielding a full IQ score of 60. His performance was considerably better on tasks which didn't require verbalization, as might be expected. One sub-test specifically measuring acuteness of observation elicited an unusually high score, suggesting that he is much more alert to the details of life around him than he appears on the surface.

Assessment of personality dynamics: Projective test findings were consistent with his intellectual level, with the exception that he exhibited a vigorous and highly active fantasy life. Primary process material gains free access to consciousness, producing hypermotility and a quality of unpredictability.

It is difficult in the records of defectives to ascertain the status of sex-role identification. In this subject, self-concept shows many indications of being an asexual one and reflects the preidentification need for a nurturant figure. There seems to be no established internal identity with either sex although, in all his role-playing activities, he is descriptively masculine. Further inferences regarding dynamics were not available because of difficulty in locating signs of ego functioning."

The habitus was normal for an adolescent male (Fig. 1). The weight was 110 pounds, height $63\frac{3}{4}$ inches, span 64 inches, measurements crown to pubis $29\frac{1}{4}$ inches and pubis to sole $34\frac{1}{2}$ inches. Although the penis was well developed, the testes were small; the right testis measured $1.9\times1.3\times1.3$ cm., and the left $2.2\times1.6\times1.3$ cm. There was slight gynecomastia bilaterally, with palpable gland tissue.

In buccal smears stained with thionin, 42 per cent of nuclei contained 2 typical masses of sex chromatin (Fig. 3a), and a single mass was present in an additional 33 per cent of the nuclei. In the spinous cell layer of a skin biopsy specimen, after staining with hematoxylin-eosin and by the Feulgen method, the sex chromatin was duplicated (Fig. 3b) in 51 per cent of the nuclei and occurred singly in a further 44 per cent. In 500 neutrophil leukocytes, 3 neutrophils with a drumstick nuclear appendage were found. The Arneth Index was 2.3.

In bilateral testis biopsy specimens (Fig. 2), a proportion of the tubules were represented by irregular, hyaline masses that sometimes contained a slit-like lumen without epithelium. Other tubules were small, with moderate thickening of the lamina propria, and were filled with cells that had oval or irregularly shaped nuclei. Germ cells could not be identified. The Leydig cells were arranged in larger aggregates than in the normal testis. The sex chromatin was duplicated in 55 per cent (Fig. 3c), and occurred singly in 40 per cent, of the Leydig cells. Nuclei within the seminiferous tubules were much less satisfactory for the study of structural detail. However, some were clearly chromatin-

TABLE 1. LABORATORY DATA

Tests	Case 1	Case 2
1. Gonadotropin excretion	>30 <50 rat uterine units	>30 <50 rat uterine units
2. 17-Ketosteroid excretion	5.9 mg./24 hrs.	3.9 mg./24 hrs.
17-Ketosteroid excretion after admin. of 25 units of ACTH	11.1 mg./24 hrs.	8.6 mg./24 hrs.
17-Hydroxycorticoid excretion	4.8 mg./24 hrs.	2.9 mg./24 hrs.
17-Hydroxycorticoid excretion after admin. of 25 units of ACTH	10.5 mg./24 hrs.	14.9 mg./24 hrs.
3. Thyroid function tests:		
BMR	−27% (pulse, 68/min.)	−29% (pulse, 58/min.)
serum PBI	5.3 μg./100 ml.	5.9 μg./100 ml.
thyroidal 24-hr. I^{131} uptake	6% of dose	12% of dose
thyroidal 48-hr. I^{131} uptake after admin. of 4 units of TSH	12%	14%
4. Liver function tests:		
serum bilirubin	0.6 mg./100 ml. (direct, 0.0 mg.; indirect, 0.6 mg.)	0.8 mg./100 ml. (direct, 0.4 mg.; indirect, 0.4 mg.)
bromsulphthalein excretion	3.2% retention after 45 min.	3.0% retention after 45 min.
thymol turbidity and flocculation (2 tests)	10 units/++; 6 units/++	9 units/++; 11 units/++
cephalin cholesterol flocculation (2 tests)	+++; +++	+++; +
5. Glucose tolerance test	see text	normal
6. Serum nonprotein nitrogen	19 mg./100 ml.	23 mg./100 ml.
7. Serum cholesterol	111 mg./100 ml.	175 mg /100 ml
8. Serum electrolytes		
Na	144 mEq./L.	142 mEq./L.
K	4.3 mEq./L.	4.4 mEq./L.
Cl	104 mEq./L.	107 mEq./L.
Ca	10.4 mg./100 ml.	9.2 mg./100 ml.
P	4.7 mg./100 ml.	5.3 mg./100 ml.
9. Electrophoresis of serum proteins		
protein	7.70%	7.30%
albumin	4.97%	4.52%
alpha-1 globulin	0.26%	0.23%
alpha-2 globulin	0.78%	0.64%
beta globulin	0.59%	1.75%
gamma globulin	1.10%	1.16%
albumin-globulin ratio	1.83:1	1.63:1

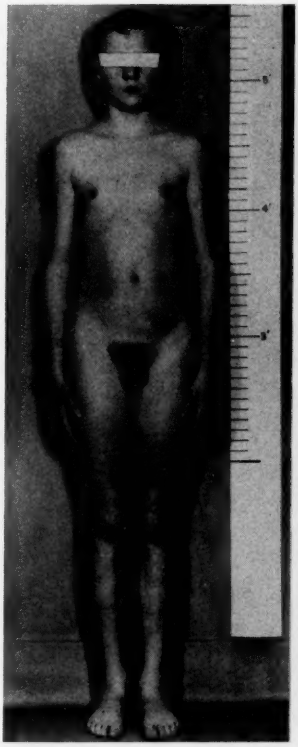


FIG. 1

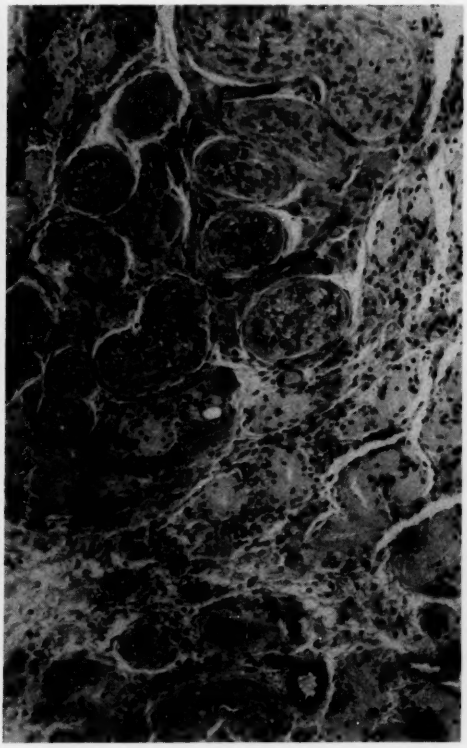


FIG. 2

FIG. 1. Case 2.

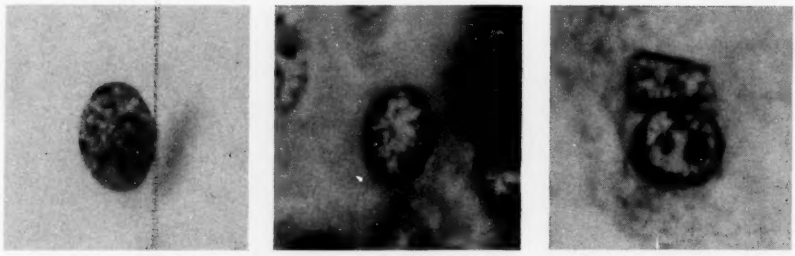
FIG. 2. Section of testicular biopsy specimen from Case 2. The histopathologic picture is characteristic of that found in the chromatin-positive Klinefelter syndrome. (Hematoxylin-eosin, × 125.)

positive and 2 masses of sex chromatin were present in a few nuclei. Aside from the duplicated sex chromatin, the histopathologic structure of the testes was characteristic of that in the chromatin-positive Klinefelter syndrome and presumably resulted from regressive changes that occurred in early puberty.

Roentgenograms of the skull and chest were normal. The electrocardiogram showed an abnormally tall R wave in lead V_1; the electroencephalogram was within normal limits. The results of laboratory tests are shown in Table 1.

THE KARYOTYPES

Chromosome analyses were carried out at the University of Western Ontario, London, using leukocytes of peripheral blood and bone marrow cells. Parallel studies were made at the Babies' Hospital, Columbia-Presbyterian Medical Center, New York, starting from skin biopsy speci-



a b c

FIG. 3. Duplication of the sex chromatin in nuclei of cells from Case 2: **a**) buccal smear stained with thionin, **b**) skin biopsy, and **c**) Leydig cell, stained with hematoxylin-eosin, × 1800.

mens that were sent from London to New York in Eagle's synthetic medium containing 1 part in 10 of human cord serum; and at the Oak Ridge National Laboratory, utilizing cells from skin cultures.

The technic of obtaining metaphase plates from leukocytes was adapted from procedures used by Jacobs (15), Miller (16) and Moorhead *et al.* (17). Modifications of their methods included the use of carbol fuchsin as a chromosome stain. The bone marrow method was that of Ford, Jacobs and Lajtha (18). Specimens of skin were established in tissue culture by the method of Grumbach, Morishima and Chu (19), using a chick plasma clot technic similar to that described by Harnden (20). Eagle's synthetic medium containing 10 per cent of pooled human placental serum was used as the nutrient medium. A sufficient outgrowth of "fibroblast-like" cells was usually obtained after incubation of the cultures for two to three weeks. The cells were subcultured on glass slides contained in Leighton tubes. Following treatment with colchicine (10^{-6} Gm./L.) and a hypotonic sodium citrate solution (0.7 per cent), temporary and permanent slides were prepared for examination according to the method of Chu (21).

Case 1

The chromosomes were counted in 54 metaphase plates obtained from leukocytes (Table 2). Forty-six cells contained 48 chromosomes, 7 cells showed 47 chromosomes and 1 cell had 49 chromosomes. The chromosome "missing" from the cells with 47 chromosomes differed from one cell to another; some of these counts were perhaps the result of loss of a chromosome in the final steps of making the preparations. The count of 49 in one cell was clearly an artefact caused by the breakage of a chromosome at its centromere. Thus, the chromosome number observed in leukocytes was 48, or 2 in excess of the normal diploid number for man.

TABLE 2. CHROMOSOME ANALYSIS (CASE 1)

Tissue	Chromosome number			Sex chromosome constitution
	47	48	49	
Blood	7	46	1	XXXY
Skin; 1st biopsy	1	25		XXXY
Skin; 2nd biopsy	1	9		XXXY
Totals	9	80	1	

A karyotype analysis was performed for 5 metaphase plates (Fig. 4). There were 5 small acrocentric chromosomes, including the Y chromosome. The 2 chromosomes that were extra to the normal diploid complement were within the chromosome 4–7 range and usually similar to chromosome 6 in length; their centromeres were submedian. Considering also that the sex chromatin was duplicated and that the patient had a gonadal abnormality, the extra elements were almost certainly X chromosomes, giving a leukocyte karyotype of 44 autosomes and an XXXY sex chromosome complex.

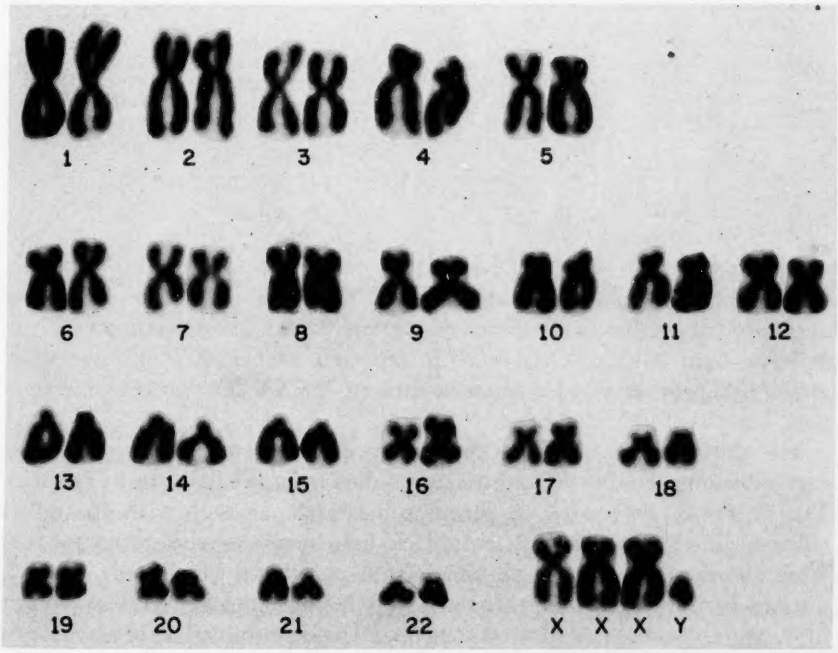


FIG. 4. The karyotype, containing a normal set of autosomes and an XXXY sex chromosome complex, constructed from a metaphase plate containing 48 chromosomes and derived from the leukocytes in Case 1.

The chromosomes in 36 cells in metaphase were counted from cultures established from 2 skin biopsy specimens (Table 2). Of these, 34 cells contained 48 chromosomes. The chromosomes in a large proportion of these cells were carefully analyzed and several karyotypes were made from photomicrographs. All karyotype analyses showed 22 normal pairs of autosomes and an XXXY sex chromosome constitution. The three X chromosomes were clearly identified in all karyotypes.

Case 2

Chromosome counts were made on 41 metaphase plates of leukocytes (Table 3). Thirty-eight cells contained 48 chromosomes, one had 47, one <46 and one 49, chromosomes. Technical artefacts could have accounted for the few counts other than 48. Karyotypes were constructed for 9

TABLE 3. CHROMOSOME ANALYSIS (CASE 2)

Tissue	Chromosome number					Sex chromosome constitution
	<46	46	47	48	49	
Blood	1		1	38	1	XXXY
Bone marrow		1	1	35		XXXY
Skin; 1st biopsy			1	64		XXXY*
Skin; 2nd biopsy				4		XXXY
Skin; 3rd biopsy		1	2	50		XXXY
Skin; 4th biopsy				15		XXXY
Totals	1	2	5	206	1	

* See text.

metaphase plates (Fig. 5). There were 5 small acrocentric chromosomes, one of them being the Y chromosome. The 2 chromosomes above the normal complement had submedian centromeres and approximated chromosome 6 in length. They were interpreted as being X chromosomes, giving a karyotype of 44 autosomes and an XXXY sex chromosome complex, like that found in Case 1.

The chromosomes in 37 metaphase figures were counted in preparations derived from a sample of bone marrow taken from the iliac crest (Table 3). There were 35 cells with 48 chromosomes and one each with 46 and 47 chromosomes. Analysis of 3 metaphase figures produced karyotypes that were identical with those obtained from peripheral blood cells. In cells obtained from the second, third and fourth skin biopsy specimens, counts of 72 metaphase plates showed that 69 of them contained 48 chromosomes (Table 3). There were 6 medium-sized and 5 small acrocentric chromosomes. Eight karyotype analyses yielded identical results—22 pairs of normal autosomes and an XXXY sex chromosome complex.

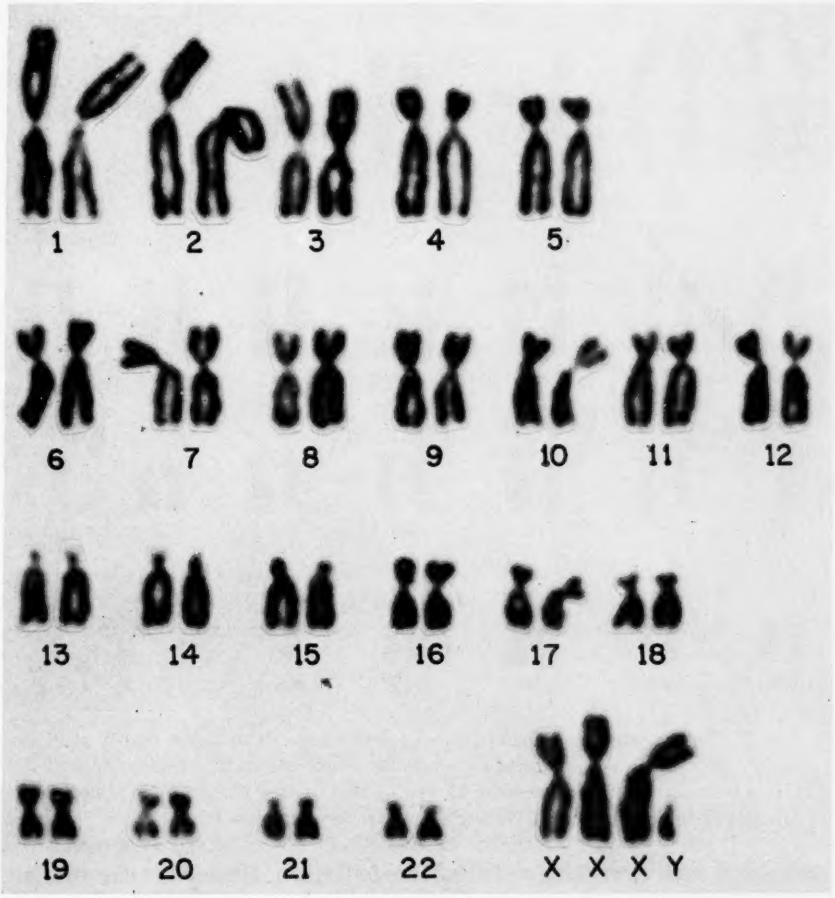


FIG. 5. The karyotype, containing an XXXY sex chromosome complex in addition to a normal complement of autosomes, constructed from a metaphase plate containing 48 chromosomes in preparations derived from the leukocytes in Case 2.

The conclusion that these 2 patients had a hyperdiploid complement of 48 chromosomes that included an XXXY sex chromosome complex was based on counting the chromosomes in 240 metaphase plates, careful visual analysis (including camera lucida drawings) of 115 metaphase plates, and the construction of karyotypes from photomicrographs for 30 metaphase plates.

Results obtained from the first biopsy are of considerable interest to students of chromosome behavior, although they are apparently not

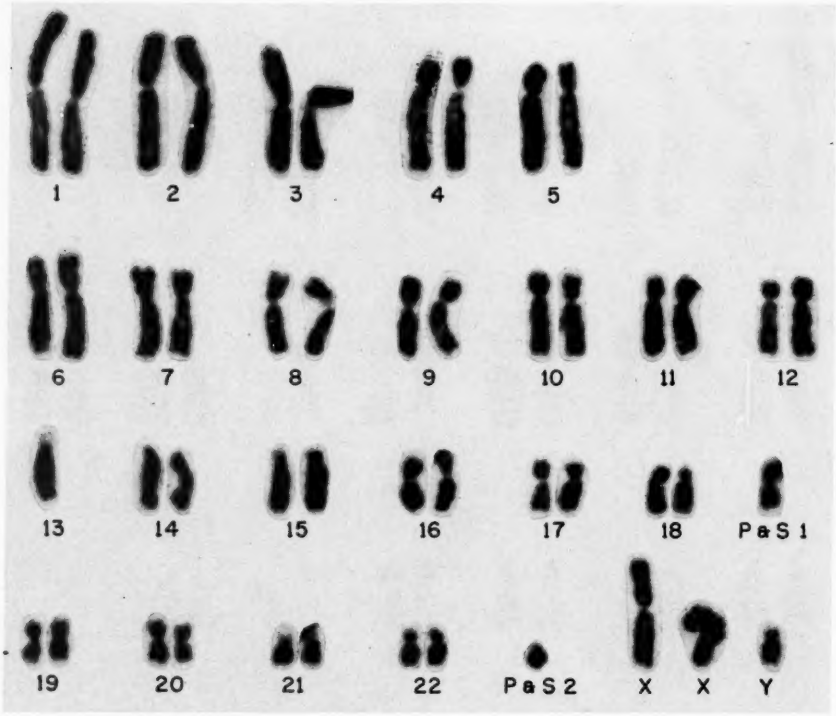


FIG. 6. The karyotype obtained from cells derived from 1 of 4 skin biopsy specimens from Case 2. P&S 1 was thought to have been derived from an X chromosome, and P&S 2 from a member of the chromosome 13 pair. These further chromosome abnormalities apparently arose *in vitro* in a slow-growing culture.

connected with the biology of the Klinefelter syndrome. The chromosome number was 48 in 64 out of 65 metaphase plates, but analysis of many cells showed a karyotype (Fig. 6) that differed from the chromosome pattern that had been well established in all other specimens from this patient. There were 5 medium-sized acrocentric chromosomes belonging to the 13–15 group, of which only one possessed a large satellite. Six short, acrocentric chromosomes were present, 5 of them belonging to the group 21–22 and Y. The other small chromosome (P&S 2) had a terminal centromere, was distinctly smaller than chromosome 22, and had no satellite. There was another abnormal chromosome (P&S 1) which approximated chromosome 18 in length and had a submedian centromere. Thus, the chromosome complement included a single chromosome 13, two abnormal chromosomes (P&S 1 and P&S 2) and an XXY sex chromosome constitution. P&S 1 was interpreted as representing an X chromosome in which a

segment from each arm had been deleted. Chromosome P&S 2 was regarded as a fragment of a chromosome 13, the major portion of which had been lost.

The same chromosome aberration was found in all of the metaphase plates analyzed from the only subculture that grew successfully from this particular skin biopsy specimen. There is a strong possibility that chromosomes P&S 1 and P&S 2 were a consequence of partial chromosome deletions in a single cell. All of the cells in metaphase studied from this particular subculture may have originated from a cell line derived from this single abnormal cell that had acquired striking mitotic activity. In any event, these findings seem to indicate the importance of studying cells cultured from more than one biopsy specimen or from parallel cultures of a single specimen, when an unusual karyotype is found.

DISCUSSION

The findings in these 2 patients introduce several problems, of which the most difficult is perhaps the origin of the XXXY sex chromosome complex. Several alternatives are theoretically possible.

(*a*) The complex may have arisen through non-disjunction of an X chromosome in the first cleavage division of an XXY zygote that would, under other circumstances, have given rise to the typically chromatin-positive Klinefelter syndrome. The initial chromosomal error would then have been one of meiotic non-disjunction of an X chromosome during parental gametogenesis, an XY-bearing sperm fertilizing a normal ovum or, perhaps more likely, a Y-bearing sperm fertilizing an ovum with two X chromosomes (22, 23). Starting with an XXY zygote, mitotic non-disjunction of an X chromosome in the first cleavage division would produce a blastomere with an XXXY complex and one with an XY complex. The XY blastomere might conceivably find expression in extra-embryonic tissues or in tissues other than those available for sex chromatin tests and chromosome analysis.

(*b*) A mother with XXX sex chromosomes in somatic cells and, presumably, oögonia could produce an XXX-bearing ovum through meiotic non-disjunction, resulting in an XXXY zygote on fertilization by a Y-bearing sperm. Females with an XXX complex in somatic cells have been described, and they may be fertile (24–27). The Western Ontario authors of the present paper have under investigation a 60-year-old female with mental retardation and schizophrenia. Her cell nuclei show duplicated sex chromatin and an XXX sex chromosome complex; her hospital record states that she has had 9 pregnancies.

An XXY Klinefelter subject could also, in theory, produce XXY sperm through meiotic non-disjunction and participate in the formation of an

XXXY zygote. According to experience to date, however, the XXY male is infertile.

(*c*) Non-disjunction in both reductional and equational divisions of gametogenesis could produce an XXX ovum and XXY sperm. Fertilization of an XXX ovum by a Y-bearing sperm, or of a normal ovum by an XXY-bearing sperm, would then produce a zygote with an XXXY sex chromosome complex.

(*d*) Meiotic non-disjunction of an X chromosome in both parents could, in theory, result in an XXXY zygote if an XX ovum were fertilized by an XY sperm. The statistical chance of such an occurrence is remote and this alternative is much more difficult to accept than a succession of chromosomal errors in the same cell lineage.

It has not been possible to carry out sex chromatin tests or chromosome analyses on the mothers of these patients to test alternative (*b*), but Ferguson-Smith, Johnston and Handmaker (8) found normal chromatin-positive nuclei in the mothers of two XXXY males. An acceptable explanation for the presence of an XXXY complex in somatic cells must await the results of further studies.

In the light of the identical karyotypes obtained for Case 2 from blood, bone marrow and 3 separate skin biopsy specimens, it seems certain that the unusual chromosomal aberration observed in cells derived from the first skin biopsy specimen arose during growth of the cells in tissue culture. It is noteworthy that explants from this specimen grew poorly for the first three weeks and that the cells in only 1 out of 4 flasks could be studied further. The cell population increased rapidly after two months of culture, at which time preparations for cytologic examination were made. The occurrence of the phenomenon of karyotypic alteration in cultured cells has been observed in cultures which initially grew sluggishly and subsequently underwent a phase of rapid proliferation (28). Such a possibility, although rare, should be considered, especially when an abnormal karyotype is encountered in cells studied from a single culture. This phenomenon can be detected, as it was in the present case, by the use of multiple biopsies or a single biopsy specimen which has been subdivided and cultured in separate flasks.

The finding that male subjects with duplicated sex chromatin in a proportion of interphase nuclei bear an XXXY sex chromosome complex is of particular interest in connection with the relation between sex chromatin and sex chromosomes. Since females with duplication of the sex chromatin have an XXX sex chromosome complex (24–27), it appears that the Y chromosome may be ignored, in the present context, in discussing the relationship between sex chromatin and sex chromosomes.

The demonstrated relationship between three X chromosomes and two

masses of sex chromatin is inconsistent with the earlier view that the sex chromatin is derived from two X chromosomes that display positive heteropycnosis and are in somatic association (7, 29). But the correlation is consistent with the findings of Ohno, Kaplan and Kinosita (30, 31) that sex chromatin in the rat and chicken is formed by a single positively heteropycnotic X chromosome. If the foregoing observations apply to man, in our patients two of the three X chromosomes displayed positive heteropycnosis in a proportion of nuclei, while the other one did not. Factors that would determine whether or not an X chromosome is positively heteropycnotic in the interphase nucleus are entirely unknown. Nonetheless, the finding of 2 female patients with gonadal dysgenesis, chromatin-positive nuclei and an XO sex chromosome complex (19, 32) strongly supports the possibility that the observations of Ohno, Kaplan and Kinosita are, in fact, applicable to man.

The 2 cases described in the present report are similar in clinical findings, including testicular histopathology, to the usual form of the Klinefelter syndrome with chromatin-positive nuclei and an XXY sex chromosome complex. The male-determining property of the Y chromosome is thus emphasized, for in its presence the testes differentiate from the indifferent gonads of the early embryo, when there are either two or three X chromosomes, although the same regressive changes are present in the postpuberal testes in both instances. Recent work has shown that the Klinefelter syndrome is associated occasionally with an XXYY sex chromosome complex (33, 34), and still other variants of the complex may be discovered in patients with the syndrome. The basic requirement for seminiferous tubule dysgenesis (disregarding in this context the chromatin-negative Klinefelter subject) appears to be the presence of at least two X chromosomes in association with at least one Y chromosome.

The elevated gonadotropin and reduced 17-ketosteroid excretions (Table 1) are usual findings in the Klinefelter syndrome. The low basal metabolic rate, deficient uptake of I^{131} by the thyroid and poor response to TSH in both patients may prove to have some significance, for Barr *et al.* (14) reported similar findings in 9 of 10 Klinefelter subjects in institutions for the mentally defective. The possibility that some form of thyroid dysfunction is a fairly regular component of the syndrome is being explored. The results of cephalin cholesterol flocculation and thymol turbidity tests suggest a disturbance of liver function in both subjects, although there was no clinical evidence to support such an inference.

Acknowledgments

The contribution by the Western Ontario Group to this study was supported by Mental Health Grants (Nos. 605-5-287 and 605-5-273) of the National Health Grants

program of Canada and the D. H. McDermid Medical Research Fund. The contribution by the Columbia University participants was supported by research grants from the National Foundation and the Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md.

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ENDOCRINE STUDIES IN A CASE OF MALE PSEUDOHERMAPHRODISM: TESTICULAR FEMINIZATION

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ABSTRACT

Adrenocortical and gonadal functions were studied in a 24-year-old patient with testicular feminization. The control values for plasma 17-hydroxycorticosteroids, urinary formaldehydrogenic steroids and urinary pregnanetriol were normal. The urinary excretion of 17-ketosteroids and pituitary gonadotropins fluctuated at the upper range of normal for males, while the urinary excretion of estrogens was high for a male of comparable age. Fractionation of the urinary 17-ketosteroids revealed normal quantities of etiocholanolone, androsterone, and 11-oxygenated 17-ketosteroids, and only small quantities of dehydroepiandrosterone. The administration of ACTH resulted in the usual increase in the level of plasma 17-hydroxycorticosteroids and all urinary steroids except dehydroepiandrosterone. Administration of dexamethasone resulted in a fall in the level of plasma 17-hydroxycorticosteroids to nearly zero, but the urinary excretion of 17-ketosteroids remained appreciable and averaged 12.5 mg. per twenty-four hours. The administration of chorionic gonadotropin, while endogenous secretion of ACTH was inhibited by dexamethasone, resulted in increased urinary excretion of 17-ketosteroids, pregnanetriol and etiocholanolone. There was a slight increase in the excretion of androsterone and 11-oxygenated 17-ketosteroids. Following orchiectomy, urinary 17-ketosteroids and estrogens virtually disappeared. It is concluded that the patient's gonads were the source of appreciable quantities of estrogens and 17-ketosteroids and small quantities of pregnanetriol.

THE syndrome commonly but perhaps inappropriately termed "testicular feminization" has been recently reviewed in detail (1–3). It is a type of male pseudohermaphrodism characterized by an unequivocally feminine appearance in the postpubertal patient. Normal breasts develop at puberty but axillary hair and pubic hair are often scant or absent and menses fail to occur. The external genitalia may be normal but not infrequently the labia minora are poorly developed. Generally there is no

Received September 15, 1960.

* The Mayo Foundation, Rochester, Minnesota, is a part of the Graduate School of the University of Minnesota.

enlargement of the clitoris, and the vagina consists of a blind pouch of variable length. The uterus is usually absent, but rudimentary fallopian tubes may be present. More often an epididymis and a vas deferens are present bilaterally. The gonads may be located intra-abdominally, in the inguinal canals, or in the labia majora, and their histologic appearance simulates that observed in cryptorchid testes. The interstitial cells may aggregate in adenoma-like formations, and tumors occur with greater frequency than in normal testes.

Although the high familial incidence of this form of male pseudohermaphrodism indicates that it is a genetic disorder, the precise mode of transmission has not been determined (2, 3). Nuclear chromatin patterns in these patients are negative, and chromosomal counts have revealed a normal diploid content of 46, including a Y and an X chromosome (4, 5). Pedigrees of families with a history of the occurrence of both testicular feminization and red-green color blindness also support the concept that these individuals are genetically males (6).

The reported endocrine studies indicate that the urinary excretion of pituitary gonadotropin is usually normal or slightly elevated and that of estrogens is normal. Castration results in the development of menopausal symptoms, increased excretion of pituitary gonadotropin, and a decrease in the levels of urinary estrogens. The excretion of 17-ketosteroids is either at the upper range of normal or moderately elevated and there is some evidence that at least a part of the urinary 17-ketosteroids is gonadal in origin (7, 8).

Because of the limited information presently available regarding gonadal and adrenal function in these patients, endocrine studies were undertaken in a 24-year-old patient with testicular feminization.

REPORT OF CASE

The patient came to the Mayo Clinic on April 20, 1959, because of primary amenorrhea. The visit was precipitated by her desire to be evaluated prior to her forthcoming marriage. Birth, growth and development had been normal until the twelfth year, when the breasts began to develop in conjunction with the appearance of axillary and pubic hair. Menstruation did not occur. Her linear growth continued until approximately the age of 15, by which time the breasts had attained normal adult size and the pubic hair was normal in both quantity and distribution for an adult female. However, the amenorrhea continued. Her interests and social orientation were typically feminine and she was well adjusted in the role of a teacher.

Her general health had been excellent and her father, mother, older brother and two sisters also enjoyed good health. Both sisters, one older and one younger, were married and had normal children, all of whom were prepubertal at the time the patient was examined. The brother was 30 years of age, unmarried, and apparently normal.

The patient was an attractive, healthy-looking, young woman, 67 inches tall and weighing 150 pounds. Her general appearance and body habitus were entirely feminine.

The breasts were well developed, pubic hair and axillary hair were normal with a female escutcheon, and there was no hirsutism. The labia majora were normal, and the labia minora abortive; the size of the clitoris was at the upper range of normal. A rudimentary hymen and a blind vaginal pouch approximately 6 cm. in depth and 3 cm. in diameter were present. No uterus was palpable. High in each inguinal canal there was an oval mass measuring 4 by 3 by 2 cm. that was slightly tender to palpation. No hernia was present.

The chromatin test (buccal smear) yielded negative results. The initial urinary excretions of 17-ketosteroids, pregnanetriol, estrogens and pituitary gonadotropin were 19.3 mg., 1.5 mg., 36 R.U., and 28 R.U per twenty-four hours respectively. The results of general laboratory studies were normal.

In order to confirm the clinical diagnosis and to obtain tissue for histologic evaluation prior to the administration of hormones, a biopsy specimen was obtained from the right inguinal mass. Following a two-month period of study the inguinal gonads were removed, the pelvis explored, and the blind vaginal pouch enlarged. Grossly, both gonads resembled normal testes and there was a well developed epididymis bilaterally. No uterus or fallopian tubes were found, but a vas deferens and a rudimentary seminal vesicle were present bilaterally.

Histologically, the gonads resembled cryptorchid testes. The tubules were somewhat smaller than those of a normal man 20 years of age. The germinal epithelium was composed almost entirely of Sertoli cells. A few spermatogonia and rarely an abnormal-appearing spermatocyte could be identified. There was considerable peritubular fibrosis. The Leydig cells appeared normal. Prominent clusters of Leydig cells were present in almost every intertubular triangle, giving the impression of a superabundance of Leydig cells. However, cell counts were not made and in the human it is not possible to give any quantitative estimate of the numbers of Leydig cells by simple inspection.

ENDOCRINE STUDIES

Methods

Urinary estrogens were determined by bioassay using uterine weight in immature rats. The average excretion in the adult male in the age group 20 to 30 years is 10 rat units per twenty-four hours with a range of 3 to 30 R.U. The value for the young adult menstruating female varies with different phases of the menstrual cycle.

The method of Albert (9) was used to determine human pituitary gonadotropin (H.P.G.) in the urine. The mean value in the young adult female is 6 R.U. per twenty-four hours (range 0 to 30 R.U.), and in the male 10 R.U. per twenty-four hours (range 4 to 135 R.U.). These values correspond to 15 units of HMG-20A and to 24 units of HMG-20A per twenty-four hours, respectively. (HMG-20A is a widely used, but unofficial, gonadotropin standard.)

Plasma 17-hydroxycorticosteroids were determined by a modification of the method of Silber and Porter, as previously described (10). The mean normal value at 8 a.m. is 16 μg. per 100 ml. of plasma with a range of 9 to 32 μg., and the mean value at 4 p.m.is 8 μg. with a range of 3 to 17 μg. Urinary 17-ketosteroids were determined by the method of Callow and associates (11), with the color correction formula of Talbot and associates (12). The range for adult males is 6 to 21 mg. per twenty-four hours and that for adult females is 4 to 17 mg. Urinary pregnanetriol was determined by the procedure of Bongiovanni and Eberlein (13).

The 17-ketosteroids were fractionated after being freed from their conjugates by β-glucuronidase and by solvolysis. An aliquot of urine (200 ml.) was incubated for sev-

enty-two hours with 200,000 units of Ketodase and 20 ml. of *N* acetate buffer in order to hydrolyze the glucuronides. Another aliquot was treated according to the method of Burstein and Lieberman (14) for solvolysis of the sulfates. The 17-ketosteroids were extracted from both aliquots with ethylene dichloride. The extracts were combined and, after evaporation of the solvent, the residue was separated into the 11-oxygenated 17-ketosteroids as a group and into dehydroepiandrosterone, etiocholanolone, and androsterone fractions by paper chromatography in the system cyclohexane:5, methanol:4, water:1 (parts by volume). The areas containing 17-ketosteroids were detected by treatment of a control strip with Zimmermann reagent. The individual fractions were identified only by their mobilities in comparison with reference compounds. The 17-ketosteroids that remained at or near the starting line were considered to be the 11-oxygenated 17-ketosteroids.

The area containing a 17-ketosteroid fraction was shaken to a pulp in a glass-stoppered Erlenmeyer flask with 10 ml. of water. The mixture was filtered, the paper was washed twice with 2.5 ml. of water and once with 5 ml. of chloroform, which was then used to extract the aqueous solution. Extraction of the aqueous solution was repeated twice more with 5 ml. of fresh chloroform. The chloroform extracts were evaporated to dryness. The residues were dissolved in an appropriate amount of absolute alcohol and the Zimmermann reaction was applied to these solutions. Readings were made at 435, 520, and 605 mμ in a Coleman Jr. spectrophotometer and the Allen correction (15) applied to the readings. Eluates of control areas showed the presence of a negligible amount of material which reacted with the Zimmermann reagent. Recovery of known 17-ketosteroids subjected to chromatography and elution was 90 to 95 per cent. Equally good recovery was obtained with eluted fractions which were rechromatographed.

The same general procedures were used for normal male controls. In addition, the 11-oxygenated 17-ketosteroids were separated into three fractions in the propylene glycol-toluene system, and the dehydroepiandrosterone fraction was assayed by the Allen "blue" reaction (16) as well as by the Zimmermann reaction. The results of the former assay usually were slightly lower than the results of the Zimmermann assay.

Certain 17-ketosteroids, such as epiandrosterone and 11-ketoandrosterone which may be present in small amounts, were not identified and, if present, probably were included in other fractions. It is likely that any epiandrosterone would have been included in the dehydroepiandrosterone fraction as assayed by the Zimmermann reaction. It would not be included in the Allen "blue" assay.

Results

During the first period the plasma levels of 17-hydroxycorticosteroids were normal and exhibited the usual diurnal variations (Fig. 1). The urinary 17-ketosteroids were mildly but definitely increased for the adult female, and at the upper range of normal for the adult male. The urinary excretion of pregnanetriol was normal (Table 1). The urinary excretion of etiocholanolone, androsterone, and 11-oxygenated 17-ketosteroids was normal but the excretion of dehydroepiandrosterone was low when compared to that of normal males (Tables 2 and 3). The urinary excretion of estrogens was high for an adult male of comparable age, but the excretion of H.P.G. was within the normal range for an adult of either sex (Fig. 2).

Administration of dexamethasone (0.5 mg. orally four times a day) was

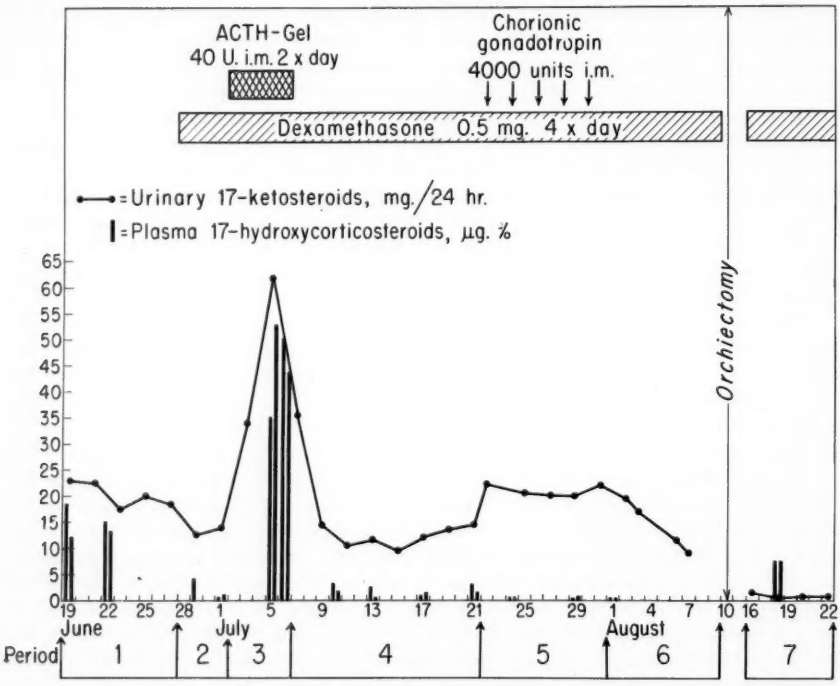

FIG. 1. Urinary 17-ketosteroids and plasma 17-hydroxycorticosteroids.

started at the beginning of the second period and continued throughout the remainder of the study in an effort to inhibit the secretion of endogenous corticotropin and thereby suppress adrenocortical function. The striking decrease in the concentration of plasma 17-hydroxycorticosteroids (Fig. 1)

TABLE 1. URINARY LEVELS OF PREGNANETRIOL

Date	Urinary pregnanetriol (mg./24 hrs.)	Treatment
June 19	1.5	None
July 4	4.8	Dexamethasone, 0.5 mg. 4 times a day, plus ACTH gel, 40 u. intramusc. twice a day
6	6.0	
12	1.0	Dexamethasone, 0.5 mg. 4 times a day
14	1.0	
24	1.7	Dexamethasone, 0.5 mg. 4 times a day, plus chorionic gonadotropin, 4000 u. intramusc. every other day
25	2.0	

TABLE 2. 17-KETOSTEROID FRACTIONS EXCRETED BY THE PATIENT (MG. PER 24 HRS.)

	Period			
	1	3	4	5
	June 26	July 4	July 14	July 28
		Dexamethasone, 0.5 mg. 4 times a day, plus ACTH gel, 40 u. intramusc. twice a day	Dexamethasone, 0.5 mg. 4 times a day	Dexamethasone, 0.5 mg. 4 times a day, plus chorionic gonadotropin, 4000 units intramusc. every other day
Dehydroepiandrosterone	0.7	0.9	2.7	0.6
Androsterone	2.6	10.0	2.3	3.0
Etiocholanolone	4.4	12.9	4.0	8.9
11-Oxygenated ketosteroids*	1.4	6.6	0.0	1.2
Total 17-ketosteroids* (crude extract)	14.1	31.4	11.2	18.2
Androsterone/etiocholanolone	0.59	0.77	0.57	0.34

* Values for 11-oxygenated ketosteroids include all material from starting line to dehydroepiandrosterone area. These values and those for total 17-ketosteroids were calculated in terms of dehydroepiandrosterone.

indicated that adrenocortical function was effectively suppressed. However, the urinary excretion of 17-ketosteroids decreased only to 12.6 and 13.5 mg. per twenty-four hours from control levels of 18.5 and 23.1 mg.

The administration of 40 units of corticotropin (ACTH gel) intramuscularly twice a day during the third period resulted in a prompt increase in the level of plasma 17-hydroxycorticosteroids and a ninefold increase in the urinary formaldehydogenic steroids (0.7 to 6.2 mg. per twenty-four hours). The urinary 17-ketosteroids more than doubled, and pregnanetriol increased (Table 1) to 4.8 and 6.0 mg. per twenty-four hours on the third and fifth days of this period respectively. The excretion of etiocholanolone, androsterone, and 11-oxygenated 17-ketosteroids increased, and the ratio of androsterone to etiocholanolone increased slightly (Table 2). The excretion of dehydroepiandrosterone did not increase. Concomitantly, the excretion of H.P.G. and estrogens increased to an average of 150 R.U. and 45 R.U. per day respectively.

When administration of ACTH was discontinued and dexamethasone alone was administered for fifteen days (period 4), the plasma 17-hydroxycorticosteroids again decreased to unmeasurably low levels while the excretion of urinary 17-ketosteroids averaged 12 mg. per twenty-four hours. The urinary excretion of pregnanetriol averaged 1.0 mg. per twenty-

TABLE 3. 17-KETOSTEROID FRACTIONS (MG. PER 24 HRS.) EXCRETED BY 3 NORMAL MALES*

	Control	ACTH	Dexamethasone	Dexamethasone plus H.C.G.
Dehydroepiandrosterone	5.1– 7.2 (6.0)†	12.9–20.1 (16.7)	0.0–0.2 (<0.1)	0.0– (0.0)
Androsterone	2.3– 5.1 (3.6)	3.5– 9.8 (5.9)	0.8–1.4 (1.1)	1.3– 3.3 (2.4)
Etiocholanolone	2.4– 4.4 (3.5)	3.5– 6.1 (4.9)	1.0–1.5 (1.3)	3.9– 4.3 (4.2)
11-Hydroxyetiocholanolone	0.4– 1.7 (0.9)	3.8– 6.2 (5.1)	0.1–0.2 (0.2)	0.2– (0.2)
11-Hydroxyandrosterone	0.3– 1.6 (0.8)	1.1– 7.6 (4.7)	0.1–0.2 (0.2)	0.2– 0.4 (0.3)
11-Ketoetiocholanolone	0.3– 1.6 (0.9)	2.0– 3.1 (2.4)	0.0–0.1 (<0.1)	0.0– 0.1 (0.1)
Total 17-ketosteroids (crude extract)	15.2–20.0 (18.0)	38.9–73.8 (52.6)	4.0–5.6 (4.7)	10.3–13.0 (11.8)
Androsterone/etiocholanolone	0.8– 1.4 (1.1)	0.8– 1.0 (1.1)	0.7–1.0 (0.8)	0.3– 0.8 (0.6)

* Fractions calculated in terms of the individual 17-ketosteroids; totals in terms of dehydroepiandrosterone.
† ( ) = Mean values.

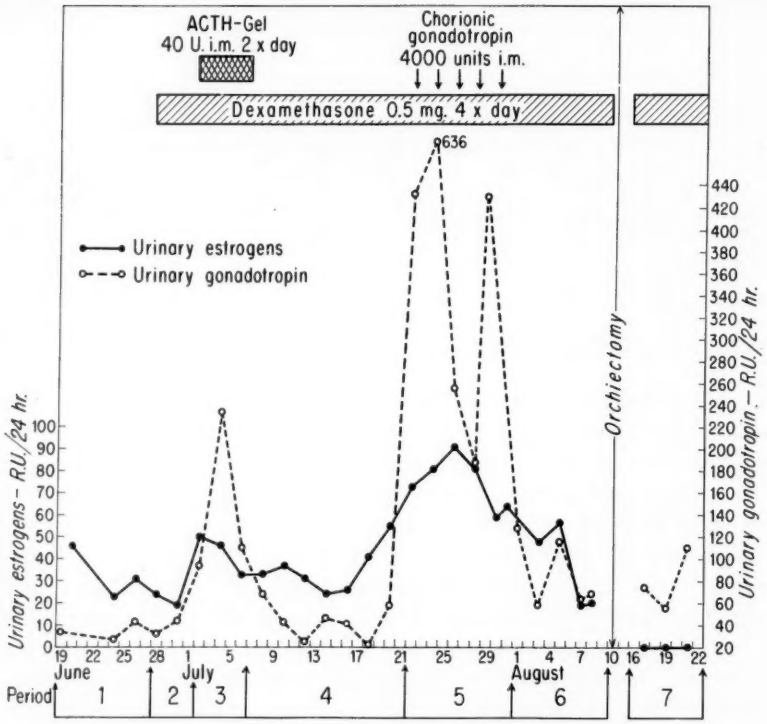

FIG. 2. Urinary estrogens and gonadotropin per twenty-four hours.

four hours during this period. The excretion of etiocholanolone and androsterone was approximately the same as it was during the control period, and the urinary 11-oxygenated 17-ketosteroids decreased to undetectable levels. Urinary excretion of H.P.G. and estrogens returned to control levels during the early part of the fourth period.

The administration of 4000 units of human chorionic gonadotropin (H.C.G.) every other day during the fifth period resulted in an increase in the urinary excretion of 17-ketosteroids of approximately 70 per cent over levels observed during the fourth period. Urinary pregnanetriol increased from 1.0 to 2.0 mg. per twenty-four hours and urinary etiocholanolone more than doubled, while there was very little change in urinary androsterone. The ratio of androsterone to etiocholanolone decreased approximately 40 per cent. The amount of 11-oxygenated 17-ketosteroids was about the same as during the control period. As expected, there was a pronounced rise in the urinary gonadotropic activity. The increase in urinary gonadotropic

activity (measured as H.P.G.) resulted from synergism between H.P.G. and the H.C.G. excreted in the urine. Urinary estrogens increased as anticipated, while the plasma 17-hydroxycorticosteroids remained suppressed.

In the sixth period, when chorionic gonadotropin was discontinued, urinary gonadotropic activity and the excretion of estrogens and 17-ketosteroids approached the values obtained during the fourth period. A week after orchiectomy the excretion of both 17-ketosteroids and estrogens was almost zero. Urinary gonadotropins rose during the latter part of the seventh period.

Approximately three months after orchiectomy and discontinuation of dexamethasone, the 24-hour urinary excretions of 17-ketosteroids, corticosteroids and pregnanetriol were normal (Table 4), and the plasma

TABLE 4. RESPONSE TO ACTH THREE MONTHS AFTER ORCHIECTOMY

Date	Urinary 17-ketosteroids (mg./24 hrs.)	Urinary pregnanetriol (mg./24 hrs.)	Urinary formaldehydogenic steroids (mg./24 hrs.)	Treatment
November 26	5.7	0.5	0.72	None
November 28	—	—	—	ACTH gel, 40 u. intramusc. twice a day
November 29	13.0	1.3	2.9	
November 30	24.2	2.4	4.8	

17-hydroxycorticosteroid levels were 25.6 and 13.6 μg. per 100 ml. at 8 a.m. and 4 p.m. respectively. The administration of 40 units of ACTH gel intramuscularly twice a day resulted in the expected increase in urinary excretion of 17-ketosteroids, corticosteroids and pregnanetriol. Since the patient was receiving exogenous estrogen, the estrogenic and gonadotropic activity of the urine was not determined.

DISCUSSION

During the second and fourth periods, while the patient was receiving a suppressive dose of dexamethasone as indicated by the very low levels of plasma 17-hydroxycorticosteroids, the average urinary excretion of 17-ketosteroids was 12.5 mg. per twenty-four hours (range, 9.6 to 14.8 mg.). However, after orchiectomy the values were less than 1 mg. per twenty-four hours. Consequently, it would appear that the gonads were the source of approximately 12.5 mg. of urinary 17-ketosteroids per day, and during stimulation with chorionic gonadotropin the gonads accounted for almost

a 70 per cent increase in urinary 17-ketosteroids to 20 mg. per twenty-four hours. The increase during administration of H.C.G. is attributed to the gonads since the plasma 17-hydroxycorticosteroid levels and the excretion of pregnanetriol showed no evidence of increased adrenal function. Less weight is given to the increase in the 17-ketosteroid fraction termed "11-oxygenated ketosteroids" because this fraction was not actually identified and because a small increase in this fraction also was noted in the patient with Addison's disease (Table 5) who had insufficient functioning adrenal tissue to show a response to ACTH. Possibly the abnormal gonads were capable of performing some 11-oxygenation. The increment in urinary 17-ketosteroids during administration of H.C.G. is comparable to that obtained by Maddock and Nelson (17), and Lubs and co-workers (7). Landau and Laves (18) noted a 72 per cent increase in urinary 17-ketosteroids in an adult male with Addison's disease during the administra-

TABLE 5. 17-KETOSTEROID FRACTIONS EXCRETED BY A MALE WITH ADDISON'S DISEASE

Fraction	Control period (mg./24 hrs.)	H.C.G. period (mg./24 hrs.)
Dehydroepiandrosterone	0.0	0.0
Androsterone	0.9	6.5
Etiocholanolone	1.0	5.4
11-Hydroxyetiocholanolone	0.1	0.1
11-Hydroxyandrosterone	0.1	0.4
11-Ketoetiocholanolone	0.1	0.3
Total, crude extract	3.9	18.8
Androsterone/etiocholanolone	0.9	1.2

tion of chorionic gonadotropin and an increase of 33 per cent in the urinary 17-ketosteroids of a group of 9 males receiving chorionic gonadotropin. Ikkos and co-workers (8) noted a 43 per cent increase in the urinary 17-ketosteroids of a patient with testicular feminization during administration of chorionic gonadotropin.

Also, it seems reasonable that the substantial amounts of urinary estrogens noted during the second and fourth periods were gonadal in origin, as they also fell to zero after orchiectomy. Urinary estrogens more than doubled during the administration of chorionic gonadotropin during the fifth period. This is similar to the observations of Ikkos and associates (8). Maddock and Nelson (17) observed even greater increments in the excretion of estrogen following the administration of chorionic gonadotropin, but in their patients the pretreatment levels tended to be low instead of elevated.

Although the increased urinary estrogens and 17-ketosteroids during the fifth period were almost certainly gonadal in origin, the changes during the third period are more difficult to interpret. The marked increase in the

concentration of plasma 17-hydroxycorticosteroids and the ninefold increase in urinary formaldehydogenic steroids in the absence of an abnormal rise in urinary pregnanetriol suggest that synthesis of hydrocortisone was probably normal. It has been previously noted that the administration of cortisone may result in an increase in the urinary excretion of gonadotropin (19) and that the administration of cortisone or similar steroids to women occasionally results in the appearance of hot flushes. Consequently, it seems possible that the increased levels of urinary gonadotropin during the third period resulted from increased adrenocortical function due to administration of corticotropin. If this is true, then the increase in urinary estrogens and part of the increase in urinary 17-ketosteroids could be gonadal in origin. Urinary estrogens and H.P.G. were determined in 2 of the 3 males who were utilized as normal controls in the evaluation of the various 17-ketosteroid fractions (Table 3), and there was an increase in both estrogens and H.P.G. during the administration of corticotropin.

The administration of chorionic gonadotropin resulted in a twofold increase in urinary pregnanetriol during a period in which adrenocortical function was suppressed. Also, postorchiectomy levels of urinary pregnanetriol were lower than control levels (Table 4). These data indicate that part of the pregnanetriol was testicular in origin, and are in keeping with the observations of others (18).

Although we have only limited information on the fractionation of urinary 17-ketosteroids in normal adults studied in a similar fashion, the data on this patient are interesting when compared with those available in the literature and with our data on 3 normal males of similar age and a 20-year-old male with Addison's disease (Tables 3 and 5). The data on excretion of dehydroepiandrosterone were more in keeping with those in the literature for females rather than with our data on 3 normal males (Table 3) or data in the literature for males (20–22). However, urinary excretions of 0.1 to 0.5 mg. of dehydroepiandrosterone per twenty-four hours have been observed in several healthy males without evidence of adrenal or gonadal disease. ACTH had little, if any, effect on the excretion of this substance by the patient but caused a pronounced increase in our group of normal males. The administration of chorionic gonadotropin to the patient, while adrenal function was suppressed with dexamethasone, was followed by a twofold increase in etiocholanolone but little increase in androsterone, resulting in a low ratio of androsterone to etiocholanolone. The administration of chorionic gonadotropin gave similar results in 1 of the normal males, but in the other 2 normal males and the patient with Addison's disease there was an appreciable increase in androsterone as well as etiocholanolone. The very low excretion of dehydroepiandrosterone, the relatively large excretion of etiocholanolone during the administration

of chorionic gonadotropin, and the comparatively large excretion of gonadal 17-ketosteroids all indicate that steroid genesis or metabolism, or both, may be significantly different from that in the control subjects.

Although the results observed in this patient did not explain the feminization, they did indicate that the gonads were the source of rather large quantities of both estrogens and 17-ketosteroids. Since the urine was not assayed for androgenic activity there is no basis for comparing the relative quantities of estrogenic and androgenic material appearing in the urine. It is quite possible that the urinary 17-ketosteroids were derived from weakly androgenic or nonandrogenic steroids. If there is a form of testicular dyfunction in which few or no androgenic steroids are produced while relatively large quantities of estrogens are being secreted, then one might speculate that a defect in testicular function is present soon after differentiation of the primordial gonad into the male gonad and that the anatomic features of the syndrome are the result of testicular dysfunction at a very early stage as well as postpubertally. Further studies, such as bioassay of the urine for androgenic activity and perfusion of surgically removed gonads, should help clarify the mechanism of feminization in similar patients.

Acknowledgment

We are indebted to Robert Smith for technical assistance in the fractionation of the urinary 17-ketosteroids.

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BILATERAL TESTICULAR TUMORS COMPLICATING CONGENITAL ADRENOCORTICAL HYPERPLASIA

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ABSTRACT

A case of bilateral steroid-secreting testicular tumors in a boy with congenital adrenocortical hyperplasia of the salt-losing type is presented. The steroidogenic activity of the testicular masses *in vitro* resembled that of abnormal adrenocortical tissue. Although large amounts of 17-ketosteroids were present in the urine, studies of the spermatic vein blood and testicular biosynthetic activity indicated that the tumors produced precursors for 17-ketosteroids but not the 17-ketosteroids themselves. There is evidence that endogenous ACTH played a role in tumor growth and secretion in this patient. It was not possible, from the studies performed, to determine whether the testicular tumors were derived from adrenocortical rest cells or from testicular interstitial cells. The importance of therapy with exogenous steroids aimed at suppressing endogenous ACTH production and urinary 17-ketosteroids is stressed.

CONGENITAL adrenocortical hyperplasia with early virilization in boys has been complicated in a few reported instances by the development of testicular tumors (1–7). The opinion has prevailed that the masses, which are histologically indistinguishable from testicular interstitial-cell tumors, develop from adrenocortical rest cells (1, 4, 7). In the present case, special studies were performed in an attempt to learn whether the secretory activity of the tumors was similar to that of testicular interstitial cells or adrenocortical tissue.

CASE REPORT

A 14-year-old white boy was first seen at Kaiser Foundation Hospital in November 1957. The diagnosis of congenital adrenocortical hyperplasia had been made within the first three months of life. The history of the early years of his illness has been included in previous publications (8, 9). The infant had been hospitalized at the age of 3 months because of acute dehydration with hyponatremia, and penile enlargement was observed. The signs of dehydration cleared following the intravenous administration of desoxycorticosterone acetate, adrenocortical extract, and physiologic sodium chloride solution.

Received September 15, 1960.

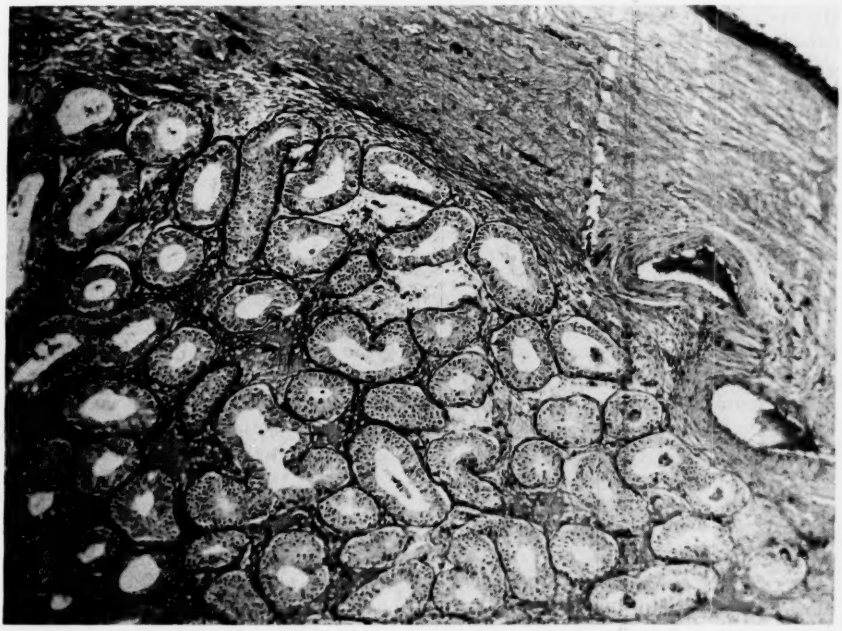

FIG. 1. Section from testicular biopsy specimen obtained when patient was 10 years old. Normal prepubertal testicular tissue. The Leydig cells appear normal. (Reduced from ×75.)

Pubic hair appeared when the infant was 2½ years old, and virilization progressed through early childhood. Starting with the second year of life, growth was excessively rapid and bone age was advanced. Epiphyseal growth centers were fused by the eighth year and growth in height ceased at 54 inches. Craving for salt began in early childhood. Between the ages of 6 months and 10 years the patient was hospitalized on four or five occasions because of addisonian crisis; therapy in each instance consisted of the administration of desoxycorticosterone acetate (DCA), physiologic sodium chloride solution, and adrenocortical extract. When the patient was 10 years old, biopsy of both testes revealed normal prepubertal testes with no evidence of spermatogenesis; normal-appearing Leydig cells were present (Fig. 1). Cortisone therapy[1] was begun (cortisone acetate, 25 mg. three times daily, orally), but the medication was not taken regularly. When the patient was 14 years old, the size of the testes appeared normal for an adolescent male. He had emotional problems, including difficulties in school and aggressive behavior, which were attributed to his short stature. On two occasions during 1957, values for the 24-hour urinary excretion of 17-ketosteroids were 12.6 and 21.6 mg. Blood pressure readings were consistently normal.

The patient was seen in the endocrine clinic of the Kaiser Foundation Hospital on November 13, 1957, at the age of 14 years and 7 months, after a mild addisonian crisis.

[1] The term "cortisone therapy" throughout the remainder of the text refers to the oral administration of cortisone acetate.

His craving for salt persisted. He was a short, muscular, adolescent boy with small hands and feet. The penis was large and the testes appeared normal in size (each about 4 cm. long) and consistency for his age. The blood pressure was 92/64 mm. Hg. Urinary 17-ketosteroid excretion was 16.2 mg., and 17-hydroxycorticosteroid excretion 7.9 mg. per twenty-four hours. The manifestations of the disease were apparently well controlled by cortisone (75 mg. daily), and this therapy was continued. Bimonthly examinations were advised.

The patient did not return until one year later, on November 6, 1958. Cortisone had been taken intermittently throughout the year, but none during the three months prior to examination. The length of each testis was now about 5 cm. and the testes were harder than before. The 24-hour urinary excretion of 17-ketosteroids had increased to 90 mg., but that of 17-hydroxycorticosteroids had diminished to 0.2 mg. These changes in excretion were attributed to lack of exogenous cortisone, and the medication (75 mg. daily in three doses) was again begun. It was taken regularly. On March 27, 1959, the 24-hour urinary excretion of 17-hydroxycorticosteroids had risen to 8.2 mg., but that of 17-ketosteroids had risen to 142 mg. One month later, the corresponding values were 18.6 mg. (17-hydroxycorticosteroids) and 95.6 mg. (17-ketosteroids). The effects of stimulation with corticotropin (ACTH) and suppression with fluorohydrocortisone were also observed (see Results and Fig. 2).

Both testes continued to enlarge rapidly in spite of continued therapy with cortisone (75 mg. daily). On June 19, 1959, *bilateral testicular biopsy and right orchidectomy* were performed. The right testis was 5 cm. long, 2 cm. wide, and weighed 55 Gm.; the left testis appeared to be the same size as the right. The entire right testis was replaced by a hard, brownish yellow tumor with fine fibrous trabeculae. The biopsy specimen from the left testis was grossly and microscopically identical with that from the right. The right testicular tumor was composed of moderately large polyhedral cells with abundant, pale cytoplasm containing blue or occasionally brown granules, and some vacuoles. These cells formed nests and masses without true acinar arrangement. The nuclei were round, of moderate size, and contained nucleoli; in a few instances, large hyperchromatic nuclei were observed. There were no crystalloids of Reinke. At one margin of the specimen, testicular tissue had been preserved, which was separated from the tumor by a circumscribed, although not encapsulated, margin. The testicular parenchyma contained tubules in which there was slight hyalinization of the basement membrane and complete absence of spermatogenesis; no nests of interstitial cells were seen. The pathologic diagnosis was bilateral interstitial-cell tumor, although it was admittedly not possible to distinguish these tumors from those composed of adrenocortical rest cells. The patient tolerated unilateral orchidectomy well (Fig. 3).

Following right orchidectomy on June 19, 1959, administration of cortisone (75 mg. daily) was discontinued. Administration of fluorohydrocortisone was begun (1.5 mg. daily, in three doses) and continued to the present. The ACTH-stimulation and fluorohydrocortisone-suppression tests were repeated, and the effects of exogenous human chorionic gonadotropin on urinary steroid excretion were studied. Less than 5 mouse units of pituitary gonadotropin was excreted daily in the urine.

Left orchidectomy was performed on September 29, 1959. The left testis had obviously increased in size during the three months since right orchidectomy; it now weighed 100 Gm. and measured 8×4.5×4 cm. (Fig. 4). The gross and histologic appearance of this tissue was similar to that on the right; the testicular mass consisted almost entirely of tumor tissue (Figs. 5, 6). At the periphery of the mass was a small area of atrophic testicular tissue consisting of a few small tubules showing no spermatogenesis. No nests of interstitial cells were seen. Following removal of the second testis, plastic prosthetic

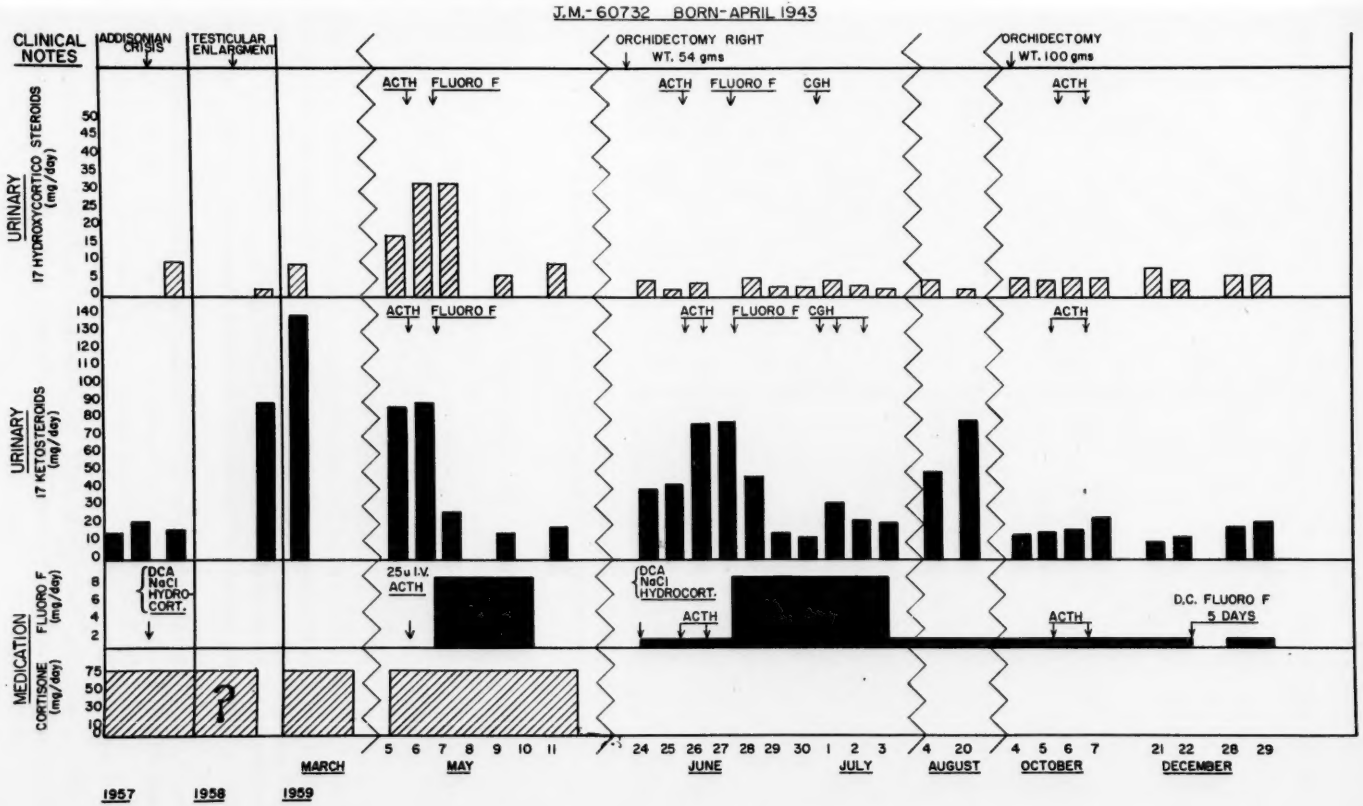

FIG. 2. Summary of urinary steroid excretion studies performed over a two-year period.

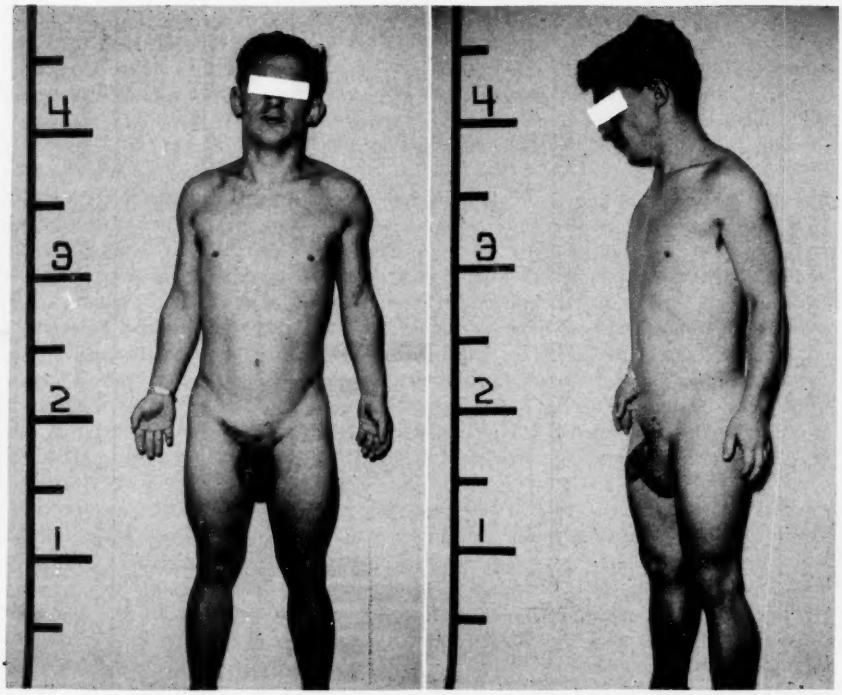

FIG. 3. Photographs of the patient at age 15 years, following right orchidectomy. Note the enlarged left testis, which was later removed.

testes were placed in the scrotum. The patient recovered uneventfully from the operation and has since been maintained with fluorohydrocortisone, 0.5 mg. three times daily. Urinary 17-ketosteroid and 17-hydroxycorticosteroid excretion values following bilateral orchidectomy were similar to those observed in 1957 prior to testicular enlargement.

METHODS

The 17-ketosteroid content of the urine was determined by the method of Drekter *et al.* (10), the 17-hydroxycorticoid content by the modified method of Reddy, Jenkins and Thorn (11), and the pregnanetriol content by the method of Cox (12). Urinary gonadotropin was determined by the method of Dekanski (13).

Tissue incubation studies and spermatic-vein blood studies are described in a separate paper (14).

Paper chromatography of urinary 17-ketosteroids was performed by the method of Brooks (15).

Special tests

ACTH stimulation. On May 6, 1959, prior to right (unilateral) orchidectomy, a standard ACTH stimulation test was performed (25 units of ACTH in 1,000 ml. of 5 per

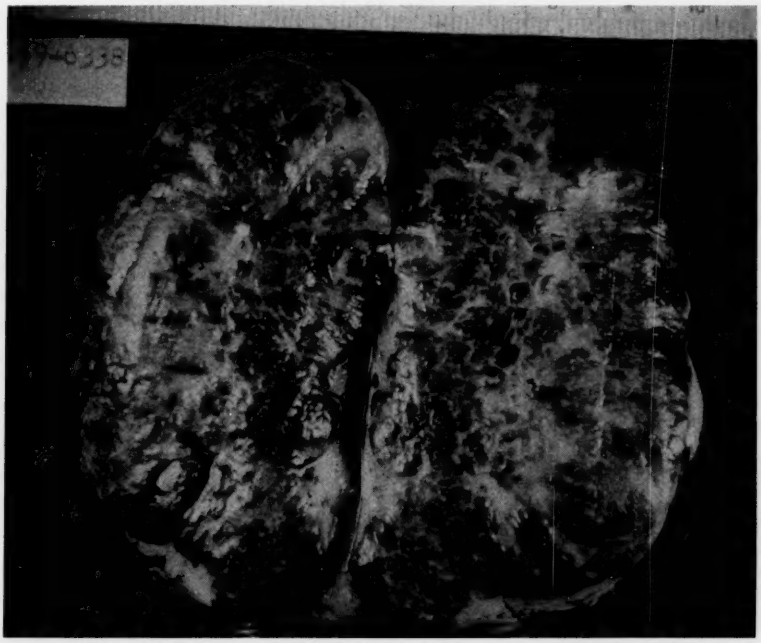

FIG. 4. The left testicular tumor, removed September 29, 1959. The weight was 100 Gm. Tumor tissue had replaced the entire testis with the exception of a small rim of atrophic testicular tissue at the periphery of the mass. (The right testicular tumor, removed June 19, 1959, was smaller than the left, but had a similar appearance.)

cent dextrose in water, given intravenously over an eight-hour period). During the test the patient continued to receive the usual dosage of cortisone (75 mg. daily). Following right orchidectomy (June 19, 1959), the standard intravenous ACTH test was performed on June 26, 1959; then a dose of 80 units of ACTH gel was given intramuscularly on June 27, 1959. On October 6, 1959 and October 7, 1959, following bilateral orchidectomy, ACTH was given intramuscularly (80 units daily for two days). During the second and third ACTH tests the patient was receiving fluorohydrocortisone (1.5 mg. daily).

Fluorohydrocortisone suppression. Prior to right (unilateral) orchidectomy, fluorohydrocortisone (8 mg. daily) was administered in addition to cortisone (75 mg. daily), as this dosage of cortisone alone had previously failed to suppress 17-ketosteroid excretion. Following right orchidectomy, on each of the six days from June 28 through July 3, 1959, an 8-mg. dose of fluorohydrocortisone was given.

Administration of chorionic gonadotropin. Beginning on June 30, 1959, while adrenocortical secretion was continuously suppressed by the administration of fluorohydrocortisone (8 mg. daily), 5,000 I.U. of chorionic gonadotropin (A.P.L.) was administered intramuscularly daily for three days.

Steroids in spermatic-vein blood; studies with ACTH. Immediately prior to left (bilateral) orchidectomy, a plastic catheter was inserted in the left spermatic vein, and a

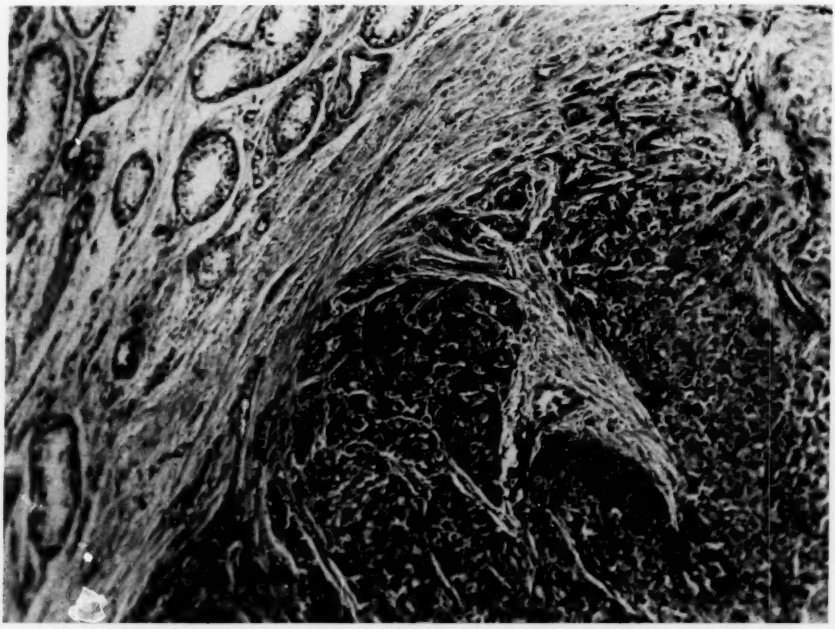

FIG. 5. Section through the periphery of the testicular tumor showing tumor cells at the right and a small area of atrophic testicular tissue at the left upper corner, with no evidence of spermatogenesis. (Reduced from ×75.)

20-ml. sample of blood was collected. An infusion of 300 ml. of 5 per cent dextrose in water containing 7.5 mg. of ACTH was given through an antecubital vein over thirty minutes; a second sample of spermatic-vein blood was collected after 6.5 mg. of ACTH had been infused, and a third sample was obtained at the completion of the infusion.

Extracts of blood from the spermatic vein were analyzed by paper chromatography in a defatting hexane-formamide system with standard solutions of testosterone, 11β-hydroxy-4-androstenedione, and 4-androstenedione (14).

RESULTS

Urinary steroid excretion (Fig. 2 and Table 1).

Effect of ACTH stimulation. Stimulation with ACTH caused only a slight increase in urinary 17-ketosteroids before orchidectomy, possibly because of the very high initial excretion. Following unilateral orchidectomy there was a pronounced increase in 17-ketosteroids upon administration of ACTH. After the second testis was removed there was a small, but definite, increase in 17-ketosteroids in response to ACTH. Urinary 17-hydroxycorticoids increased following ACTH stimulation only prior to the

first orchidectomy, and not after right and bilateral orchidectomy. The high urinary 17-hydroxycorticoid values before and after the first ACTH test, but not after unilateral and bilateral orchidectomy, may be partially explained by the fact that the patient was receiving 75 mg. of cortisone daily during the first test whereas during the subsequent ACTH tests the administered steroid was fluorohydrocortisone (1.5 mg. daily).

Effect of fluorohydrocortisone suppression. The urinary 17-ketosteroids could be suppressed by fluorohydrocortisone in large dosage (8 mg. daily) before orchidectomy, and after unilateral orchidectomy. The 17-hydroxycorticoids could be similarly suppressed.

Effect of chorionic gonadotropin. On the first day of administration of chorionic gonadotropin (twenty-eight days prior to the second orchidectomy), urinary 17-ketosteroid values doubled. On the second and third days, however, these values approximated those observed prior to treatment with chorionic gonadotropin. Chorionic gonadotropin did not demonstrably affect 17-hydroxycorticoid excretion.

17-Ketosteroid fractionation by paper chromatography (Table 2). Urine

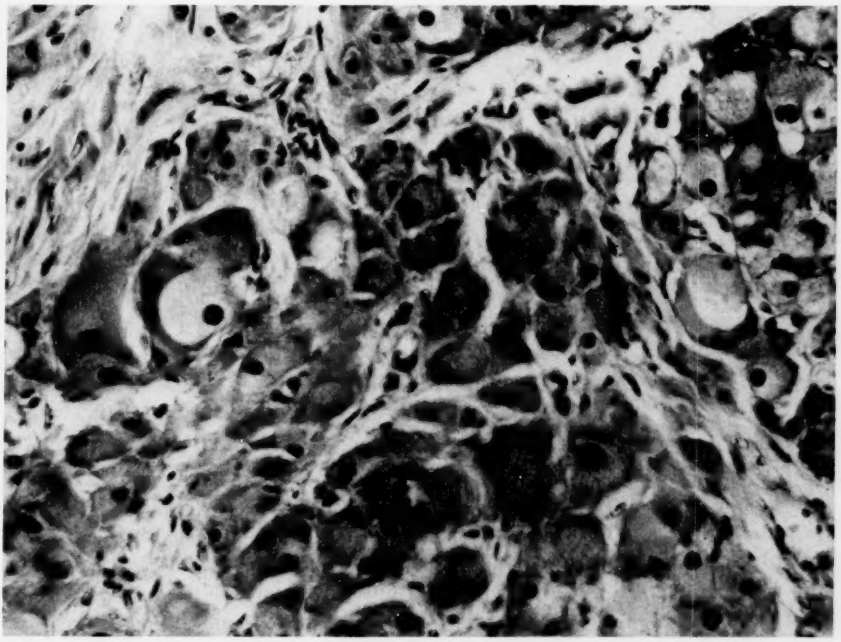

FIG. 6. High-power view of tumor tissue from the left testicular mass. No crystalloids of Reinke are seen. (Reduced from ×315.)

TABLE 1. RESULTS OF URINARY STEROID EXCRETION STUDIES PRIOR TO ORCHIDECTOMY, FOLLOWING UNILATERAL (RIGHT) ORCHIDECTOMY, AND FOLLOWING BILATERAL (LEFT) ORCHIDECTOMY

(See "Methods" section for steroid dosages during studies.)

	Prior to orchidectomy		Following right orchidectomy (June 19, 1959)		Following left (bilat.) orchidectomy (Sept. 29, 1959)	
	Before ACTH	After ACTH	Before ACTH	After ACTH	Before ACTH	After ACTH
ACTH stimulation						
17-ketosteroids (mg./24 hrs.)	95.6	103.0	38.6 43.1	75.7 79.5	14.9 15.5	15.5 25.7
17-OH-corticoids (mg./24 hrs.)	18.6	32.8	4.3 2.3	3.9	6.4 5.5	5.8 5.9
	Before fluoro-F	After fluoro-F	Before fluoro-F	After fluoro-F		
Fluorohydrocortisone suppression						
17-ketosteroids (mg./24 hrs.)	103	Day 1—26.0 Day 3—15.0 Day 4—19.0	Day 1—38.6 Day 2—43.1	Day 0—47.7 Day 1—16.7 Day 2—15.0		
17-OH-corticoids (mg./24 hrs.)	32.8	Day 1—32.0 Day 3— 5.5 Day 4— 9.0	Day 1— 4.3 Day 2— 2.3	Day 0— 6.3 Day 1— 3.4 Day 2— 3.3		
			Before C.G.	After C.G.		
Effect of chorionic gonadotropin						
17-ketosteroids (mg./24 hrs.)			Day 1—16.7 Day 2—15.0	Day 1—32.5 Day 2—21.4 Day 3—15.7		
17-OH-corticoids (mg./24 hrs.)			Day 1— 3.4 Day 2— 3.3	Day 1— 4.9 Day 2— 2.9 Day 3— 2.1		
Urinary pregnanetriol (mg./24 hrs.)	66.2		15.4		7.4	
					1 month after	3 months after
Urinary gonadotropin (mouse units per 24 hrs.)	<5		<5		5, <80	5, <80

specimen #1 (August 20, 1959) was collected following removal of the first testis; specimen #2 (December 21, 1959), after bilateral orchidectomy. The results indicated that the elevation in urinary 17-ketosteroid values was caused mainly by the 11-oxygenated group of compounds, the 11-desoxygenated group being present in approximately normal amounts.

Steroids in spermatic-vein blood; effect of ACTH

Neither 4-androstenedione, testosterone, nor 11β-hydroxy-4-androstenedione was present in the spermatic-vein blood, either before or after administration of ACTH. The 2 compounds isolated from spermatic-vein blood by means of paper chromatography were 17α-hydroxyprogesterone and 4-pregnene-17α,20α-diol-3-one. Upon administration of ACTH, the level of 17α-hydroxyprogesterone in the spermatic-vein blood increased, indicating that this compound was the main steroid produced by the tumor (14).

TABLE 2. URINARY 17-KETOSTEROID FRACTIONATION BY PAPER CHROMATOGRAPHY
(Determinations made by Bio-science Laboratories, Los Angeles, California.)

	Normal range for adult males (mg./24 hrs.)	Values in patient of present study (mg./24 hrs.)		
		Aug. 20, 1959*	Dec. 21, 1959†	Dec. 22, 1959
17-ketosteroids	9–22	79.0	13.5	
Pregnanetriol	Up to 4	15.4	9.1	7.4
11-Desoxy group:				
DHA	0–1.9	0.1	Less than 0.1	
Etiocholanolone	1.4–5.7	5.4	0.7	
Androsterone	2.0–6.0	5.6	1.6	
11-Oxy group:				
11-OH-etiocholanolone	0.5–0.9	2.7	0.3	
11-OH-androsterone	0.1–1.0	8.6	1.2	
11-Keto-etiocholanolone	0.2–1.2	3.2	0.8	
11-Keto-androsterone	0.1–0.6	3.8	0.9	

* Unilateral orchidectomy was performed June 19, 1959.
† The second testis was removed September 29, 1959.

Urinary gonadotropin excretion

Urinary gonadotropin rose from less than 5 mouse units prior to orchidectomy and after unilateral orchidectomy, to more than 5 and less than 80 mouse units, when determined one month and again three months after complete orchidectomy.

The testicular tumors; incubation studies

The results of these studies, reported in a separate paper by one of the authors (14), indicate that *in vitro* the main biosynthetic activity of the tumor was 17α-hydroxylation, and that 17α-hydroxyprogesterone was the compound produced in greatest quantity. 17-Ketosteroids were not synthesized by the tumor tissue in significant amounts. It was thought probable that, *in vivo*, the large quantities of 17-ketosteroids found in the urine while the tumor was present were being transformed in the liver from their precursor, 17α-hydroxyprogesterone, large amounts of which were synthesized by the tumor. Side-chain splitting enzyme was not produced by the tumor; this accounted for the lack of biosynthesis of testicular androgen. Small quantities of 21-hydroxylase and 11-hydroxylase were present in the testicular tumor.

DISCUSSION

The occurrence of steroid-secreting testicular tumors in a boy with congenital adrenocortical hyperplasia of the salt-losing type suggests a relationship between the adrenocortical abnormality and tumor forma-

tion.[2] Neither *in vivo* studies of the secretory activity of the tumor during the period of rapid tumor growth nor tissue incubation studies proved conclusively whether the tumors developed from "adrenocortical rest tissue" or from testicular interstitial cells. Histologically the tumor tissue resembled either adrenocortical or testicular interstitial cells. The steroidogenic activity of the testicular tumors most closely resembled the abnormal secretion of congenital adrenocortical hyperplasia and favors an adrenocortical origin of the tumors, whereas the location of the tumors in both testes favors their origin from testicular tissue. Results of the studies strongly indicated that, although the origin of the tumors was in doubt, their secretory activity and possibly their rate of growth were dependent upon the action of ACTH.

There is good evidence to indicate that ACTH is secreted in excess in untreated congenital adrenocortical hyperplasia (16–20). This excess ACTH secretion can be suppressed by the administration of cortisone or related steriods (21). Presumably ACTH secretion is greater in the salt-losing variety of the disease (16). About one third of patients with congenital adrenocortical hyperplasia exhibit excessive renal excretion of sodium and chloride, as manifested by hyponatremia and frequent addisonian-like crises. Studies (16) indicate that defective 21-hydroxylation is the intrinsic abnormality in the normotensive form of congenital adrenocortical hyperplasia whether or not renal salt excretion is increased, but in the salt-losing variety the defect is more nearly complete. It is significant in this connection that of the reported cases of congenital adrenocortical hyperplasia in which testicular tumors have developed, all have been associated with excessive renal salt excretion (3).

ACTH appears to have played a role in the development of the tumors reported here. Rapid tumor growth began while the patient probably was experiencing increased ACTH secretion, resulting from failure to take cortisone regularly. Urinary 17-ketosteroid excretion rose as the tumors increased in size, suggesting the action of a tropic hormone. These high values for urinary 17-ketosteroids could be suppressed to normal only by the administration of very large doses of exogenous steroids (75 mg. of

[2] For the sake of clarity it should be emphasized that the word "tumor," which is used to describe these masses, does not imply that the authors favor a particular tissue of origin of the masses. The nonspecific meaning of the words "tumor," "true tumor," and "neoplasm" are exemplified by the following definitions from Dorland's Illustrated Medical Dictionary (23rd Edition); other reference sources furnish similar definitions:

Tumor: 1. Swelling; morbid enlargement. 2. A neoplasm. A mass of new tissue which persists and grows independently of its surrounding structures, and which has no physiologic use.

True tumor: Any tumor produced by proliferation.

Neoplasm: Any new and abnormal growth, such as a tumor.

cortisone plus 8 mg. of fluorohydrocortisone daily). The dose of cortisone (75 mg. daily) which previously had been sufficient to suppress the urinary 17-ketosteroid levels to normal had no effect. Since massive doses of exogenous steroids were administered for only a few days, no gross suppressive effect on the rate of growth of the testicular tumor could be noted.

There is evidence that testicular masses associated with congenital adrenocortical hyperplasia can be made to shrink by administration of cortisone (1, 6, 7). In our case, pathologic examination showed that both testes were almost completely replaced by tumor tissue, only a small rim of atrophic testicular tissue remaining on each side. In our opinion, early, adequate administration of steroid will prevent the development of testicular tumors in males with congenital adrenocortical hyperplasia. In the case reported here, surgical removal seemed indicated in view of the rapid growth of the testicular tumors, absence of normal testicular tissue, and failure of the masses to shrink during the administration of 75 mg. of cortisone daily.

The close correlation between the rapid rate of tumor growth and the increase in urinary 17-ketosteroid excretion, the fall in urinary 17-ketosteroid excretion following removal of the tumors, and the effects of fluorohydrocortisone suppression and ACTH stimulation (see Fig. 2, Table 1, and Results) all constitute evidence that the testicular tumors in this boy were ACTH-dependent.

The tissue incubation studies showed that the steroidogenic activity of the testicular tumors most closely resembled the abnormal secretion of congenital adrenocortical hyperplasia. Neither hydrocortisone, the end-product of normal adrenocortical activity, nor testosterone or androstenedione, the normal testicular steroids, was synthesized by the tumor incubates. The chief steroid synthesized by the testicular tumors was 17-hydroxyprogesterone. There was no evidence of the presence of the testicular side-chain splitting enzyme in the tumor tissue. These findings might suggest that the tumors were derived from adrenocortical rest cells, an assumption that would require the presence of abnormal adrenocortical tissue in both testes. This is an attractive and popular theory but there is a lack of objective information about the incidence of so-called "adrenocortical rest cells" in testicular tissue. The "unorganized state of the literature" on this subject, referred to by Nelson (22) in 1939, is no clearer today. One sees reference in the literature to adrenocortical tissue occurring "not uncommonly" outside the adrenal glands, and Gardner *et al.* (4) reported tissue resembling adrenocortical cells in a number of sites outside the adrenal gland in a boy with congenital adrenocortical hyperplasia in whom testicular tumors developed. Before definite conclusions can be drawn about the derivation of these testicular tumors, there is need for

further studies aimed at determining the incidence of adrenocortical cells in testicular parenchyma both in normal males and in those with congenital adrenocortical hyperplasia. Such studies are impeded by the inability to distinguish between adrenocortical and testicular interstitial cells by currently available techniques.

In order to support a testicular interstitial-cell origin of the tumors occurring in congenital adrenocortical hyperplasia of the salt-losing variety, one must hypothesize that the secretory activity of the testicular interstitial cells under prolonged ACTH stimulation becomes similar to that of abnormal adrenocortical tissue. In some instances, the testicular interstitial cells may respond to endogenous ACTH by increased secretory activity, by hyperplasia, and finally by tumor formation. A few facts can be marshalled in favor of this hypothesis. There is evidence to suggest that testicular cells can synthesize 17-hydroxyprogesterone, the main steroid produced by the tumors in this case; pregnanetriol, considered to be of testicular origin, has recently been demonstrated in the urine of normal men (23). The enzyme, 21-hydroxylase, present in small amounts in the tumor tissue and previously considered to be specifically an adrenocortical enzyme, has been shown to be present in normal rat and mouse testes (24, 25). Another supposedly specific adrenocortical enzyme, 11-hydroxylase, was present in our case, but has not been demonstrated in normal testicular tissue as far as we are aware (14). However, Savard *et al.* (26) recently reported the case of a virilizing unilateral testicular interstitial-cell tumor in a human being; 11-hydroxylase activity was present in this tumor. There was no evidence of congenital adrenocortical hyperplasia. Crystalloids of Reinke were present in the tumor cells, and side-chain splitting enzyme activity was indicated by the ability of the tumor tissue to synthesize androstenedione and testosterone *in vitro*. Although their case appeared to be a classic example of testicular interstitial-cell tumor, the presence of 11-hydroxylase activity led the authors to consider the possibility that the tumor arose from adrenocortical rest cells. Thus, it is apparent that even with the latest techniques in steroid biochemistry and histology it may not be possible to prove the origin of secreting testicular tumors.

In the present state of knowledge, it is feasible only to conclude that when the salt-losing defect complicates congenital adrenal hyperplasia in males, the development of testicular tumors is a potential danger, and that the biosynthetic activity of such tumors resembles that of abnormal adrenocortical tissue. In view of the important role played by ACTH in the pathogenesis of these tumors, it would seem that the high levels of ACTH should be continuously suppressed with adequate corticoid therapy

from an early age, in an attempt to prevent tumor development in the testes.

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CORRELATION OF INSULIN REQUIREMENTS WITH THE CONCENTRATION OF INSULIN-BINDING ANTIBODY IN TWO CASES OF INSULIN RESISTANCE

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ABSTRACT

A correlation between the concentration of insulin-binding antibody and the degree of insulin resistance was demonstrated in 2 patients with insulin-resistant diabetes. Both patients had high antibody titers during the acute phase of insulin resistance. The titers decreased concomitantly with a decrease in the insulin requirement. These findings suggest that in some cases insulin-binding antibodies may account for insulin resistance.

FEWER than 100 cases of insulin resistance had been reported prior to 1950 (1, 2). Recent reviews have shown that this condition is more common than previously believed (3–5).

The usual criterion for the diagnosis of insulin resistance, set by Martin *et al.* (6), is the requirement of at least 200 units of insulin per day for at least two days. It is probable, however, that an insulin requirement as low as 100 units daily constitutes a significant degree of insulin resistance, in view of the experience with completely depancreatectomized subjects (7, 8) and the great majority of diabetic subjects.

Serum from insulin-resistant patients neutralizes the hormonal effect of insulin *in vivo* (9–13). In these cases the insulin resistance was thought to be on the basis of immunity. The insulin-neutralizing factor, believed to be an antibody, was found in the gamma-globulin fraction of the serum (14–16), whereas skin-sensitizing insulin antibody was localized in the beta-globulin fraction (17). It is now recognized that, although insulin is a weak antigen, insulin-binding antibodies develop within six to eight weeks in virtually all persons receiving the hormone continuously (18). However, the maximal insulin-binding capacity of the serum of most insulin-treated patients is less than 10 units per liter (18) compared with 60 units per liter or more in insulin-resistant patients (3, 5, 13, 19, 20). The 2 patients presented here demonstrate both the clinical and immunologic qualifications for a diagnosis of insulin resistance.

Received April 27, 1960.

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METHODS, CASE REPORTS AND RESULTS

The methods employed in the labeling of insulin with I^{131} and the quantitative demonstration of insulin-binding by antisera were those described by Berson and Yalow (5, 18, 20).

Case 1

J.P. (P.H. #132-16-22), a 48-year-old white female, was admitted to the Presbyterian Hospital, New York City, on May 3, 1957, for the management of insulin-resistant diabetes. Sixteen months before admission a chronic ulcer of the left foot had developed and had progressed to gangrene and osteomyelitis. Control of the diabetes became increasingly difficult, and inflammation of the foot continued in spite of surgical drainage and antibiotic therapy. On the day of transfer to Presbyterian Hospital the patient had been given 14,000 units of insulin and sodium bicarbonate for the treatment of acidosis.

The physical findings on admission included extensive edema of both legs and a draining infected lesion between the first and second toes of the left foot.

Laboratory findings on admission: The leukocyte count was 16,000 per cu. mm., hematocrit 27 per cent, and erythrocyte sedimentation rate 90 mm. per hour. The level of blood glucose was 179 mg. per 100 ml.; blood urea nitrogen, 20 mg. per 100 ml.; serum sodium, 160 mEq. per liter; potassium, 1.6 mEq. per liter; carbon dioxide, 34.4 mEq. per liter; albumin 2.6 Gm. per 100 ml.; gamma-globulin, 2.2 Gm. per 100 ml., and other globulins normal. No acetone was present in the serum. Urinalysis showed glucose 4 plus, no acetone or diacetic acid, albumin 2 plus and many leukocytes per high-power field.

Roentgenograms of the left foot were compatible with a diagnosis of osteomyelitis of the distal end of the first metatarsal and the proximal portion of the first proximal phalanx.

Management of her condition was difficult initially but two weeks after admission she was able to undergo amputation of the left great toe and the first metatarsal. Chloramphenicol and streptomycin were administered and the wound healed well.

A chronic *Staphylococcus aureus* infection of the urinary tract responded to treatment with furadantin and sulfisoxazole.

Insulin dosages are shown in Figure 1. Administration of 14,000 units of insulin before transfer to the hospital was followed by hypoglycemia twenty-four hours later. A pattern then developed of alternating acidosis (increased insulin requirement) and hypoglycemia; this was repeated several times during the first four weeks of hospitalization. The requirement for insulin diminished gradually during the next ten weeks. The patient was receiving 80 units of NPH insulin when she was discharged from the hospital.

Tolbutamide, 3 Gm. per day, was given on three separate occasions (Fig. 1). The insulin dosage was decreased from 180 to 80 units daily during the last period of tolbutamide therapy. Insulin requirements had decreased, however, before tolbutamide was given.

Maximal insulin-binding capacities for the 3 plasma samples are shown in Figures 1 and 2. The first two samples, taken when insulin was being

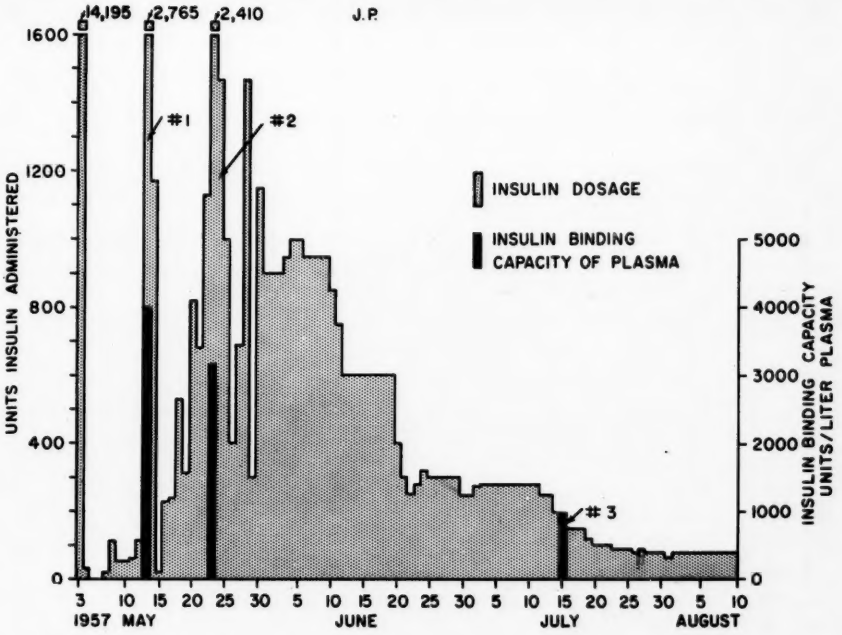

FIG. 1. *Case 1.* Daily dose of insulin (left ordinate) and maximal insulin-binding capacities (right ordinate). Tolbutamide, 3 Gm. daily, was given on June 16, 17, 26, 27, 28 and July 9 through 26.

administered irregularly and in inordinately high dosages, showed binding capacities of 4,000 and 3,500 units of insulin, respectively, per liter of undiluted plasma. The third sample was withdrawn during the recovery phase; the binding capacity was 1,000 units per liter of undiluted plasma.

Case 2

L. J. (J.C.D.H. #9033), a 48-year-old Negro female, was transferred to the Jewish Chronic Disease Hospital, Brooklyn, for management of diabetes. Her past medical history had been uneventful, although her alcohol intake had been excessive for the previous ten years. Diabetes had been diagnosed one year before admission. At that time she had been hospitalized elsewhere for treatment of acidosis, and when discharged was taking 55 units of NPH insulin per day. A second period of hospitalization had been necessary four months before the present admission. Her daily insulin requirement had risen gradually to 120 units of protamine zinc insulin. Nine days after discharge she was readmitted in diabetic coma. She was given 1,100 units of regular insulin to combat acidosis. Initially, the diabetes remained uncontrolled with 600 units of insulin daily, but by the time of admission to the Jewish Chronic Disease Hospital the insulin dosage had been reduced to 180 units (90 units twice daily).

On physical examination nothing abnormal was noted except a firm, nontender liver which was palpable 6 cm. below the right costal margin.

Laboratory findings: The fasting blood sugar levels ranged from 67 to 380 mg. per 100 ml. The concentration of serum albumin was 3.8 Gm. per 100 ml. and of globulins, 3.2 Gm. per 100 ml. Thymol turbidity was 3 plus and the serum alkaline phosphatase level was 24.5 King-Armstrong units; both values later became normal. The results of other routine liver function tests were normal. A urine culture yielded a growth of *Staphylococcus aureus*.

An intravenous pyelogram revealed calcification of the left adrenal and pancreas; the right kidney could not be visualized.

The dosages of insulin are shown in Figure 3. The diet remained constant and contained 2,500 calories and 250 Gm. of carbohydrate per day. This

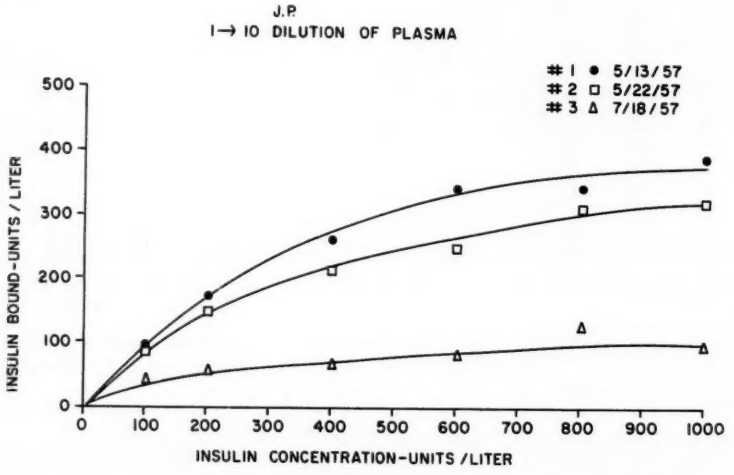

FIG. 2. *Case 1.* The amount of insulin bound by 3 plasma samples, as a function of the concentration of crystalline beef insulin.

patient, like Patient J.P., had alternating episodes of acidosis and hypoglycemia during the first month of hospitalization. Prednisone, 30 mg. per day, was administered from September 9 until November 4, 1958. The average daily dose of insulin declined from 400–500 units to 130 units, but prednisone was discontinued after persistent polyuria and glycosuria appeared. Control of the diabetes was only fair with 100 to 200 units of insulin per day during the next three months. A course of tolbutamide therapy was ineffective. However, after eight weeks of treatment with DBI (phenformin) the insulin requirement had diminished. At the time of this report, the patient was receiving a daily dose of 90 units of NPH insulin alone.

The maximal insulin-binding capacities of 5 samples of plasma are shown in Figures 3 and 4. Before prednisone therapy was begun the insulin-

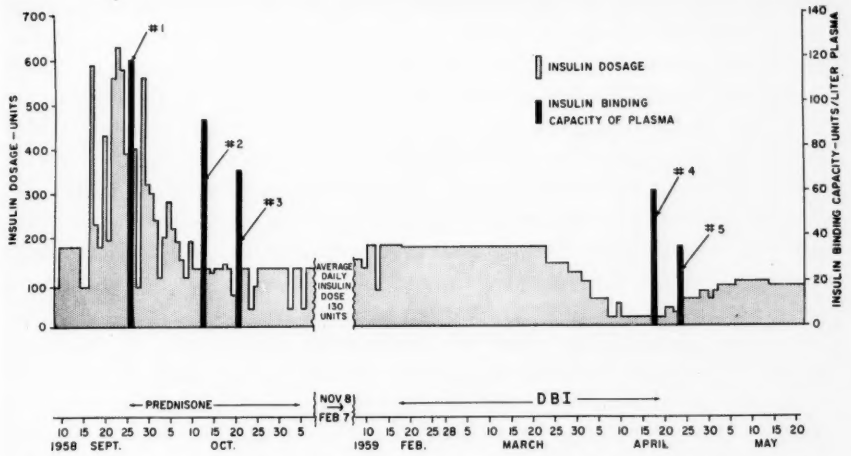

FIG. 3. *Case 2.* Daily dose of insulin (left ordinate) and maximal insulin-binding capacities (right ordinate). Prednisone, 7.5–20 mg. daily, was given from October 21 to November 4, 1958. DBI, 50–400 mg. daily, was given from February 19 to April 18, 1959.

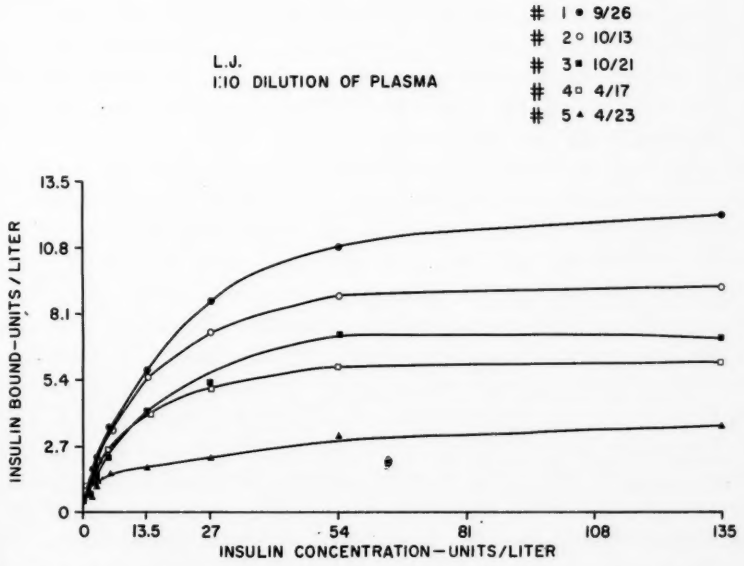

FIG. 4. *Case 2.* The amount of insulin bound by 5 plasma samples, as a function of the concentration of crystalline beef insulin.

binding capacity of the plasma was 121 units compared with 70 units toward the end of the period of steroid therapy. The maximal insulin-binding capacity of 60 units per liter of plasma noted near the end of DBI therapy declined further to 35 units after the discontinuation of DBI.

DISCUSSION

A decrease in the concentration of insulin-neutralizing antibody concomitant with the disappearance of insulin resistance has been previously demonstrated (15, 21). The methods used to illustrate a decline in antibody titer have been, at best, semi-quantitative; they have included a study of the protection offered by serum (or the gamma-globulin fraction) from insulin-resistant patients either in insulin tolerance tests (9, 15, 21) or in tests using a combination of insulin with the rat diaphragm (22). The use of insulin-I^{131} provides a more sensitive method for determining the maximal insulin-binding capacities of insulin antisera (5, 18, 20). The insulin requirement in cases of immunologic resistance is not determined solely by the concentration of insulin-binding antibody, as Berson and Yalow (5) have pointed out. Additional factors include: 1) the ratio of bound to free insulin required to control diabetes, 2) the rates of formation and dissociation of the insulin-antibody complexes, and 3) the speed of the immunologic elimination of these complexes from the circulation. Only the actual concentrations of insulin-binding antibodies are known for Patients J.P. and L.J. Figures 1 and 3 show that the maximal insulin-binding capacities were not strictly correlated with the insulin dosages although the trends for both were in the same general direction.

The increased production of insulin antibody may have been stimulated in both patients either nonspecifically by infection or by the larger dosages of insulin required because of the infection. Regarding the latter premise, it appears unlikely that antibody concentrations would be so closely dependent upon antigen dosage in patients given antigen daily for a year. Furthermore, repeated studies in one subject extending over a year have shown that discontinuation of insulin had virtually no effect upon antibody concentration (5). Although infection may increase the requirement for insulin, regardless of the presence of insulin antibodies, the extremely high requirement for insulin in our 2 patients could not be explained on this basis alone.

When high concentrations of insulin-binding antibodies are present, the large amounts of insulin administered are in part rapidly metabolized, and in part bound to the antibody. The portion of the dose bound in an antigen-antibody complex is not available to the tissues until dissociation of the complex has released the insulin. In human subjects these insulin-antibody complexes show both rapid (three to thirty minutes) and slow (two to twenty hours) half-times of dissociation (5, 12, 19, 20). This dissociation of

the insulin-antibody complexes may account for the late hypoglycemic reactions in Patient J.P. in whom, on at least one occasion, severe hypoglycemia developed twenty-four hours after the last dose of regular insulin.

Since the precise stimulus for increased insulin-antibody production is still unknown, the management must be based largely on empirical methods. Insulin resistance has been associated with a variety of disorders (1, 2, 23). Experimentally, cortisone inhibits synthesis of an antibody following specific sensitization (24) and interferes with the anamnestic response (13). Because of these theoretical considerations, either cortisone or ACTH has been used in the treatment of insulin-resistant patients. These hormones have reduced the requirement for insulin (15, 21) and, in one instance, completely eliminated the need for insulin (25). Steroid therapy in Patient L.J. caused a decrease in both the insulin requirement and the plasma insulin-binding capacity. When glycosuria reappeared the steroid was discontinued.

Interpretation of the relationship between steroid therapy and antibody concentration is difficult in our 2 patients because they were given oral hypoglycemic agents intermittently. Therefore, it is not possible to draw conclusions regarding the influence of hypoglycemic agents and insulin antibodies upon the short-term fluctuations in the insulin requirement. However, the general decline in antibody concentration that accompanied the decrease in the insulin requirement over several months suggests more than a coincidental relationship.

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APPARENT INSULIN ACTIVITY IN A FIBROSARCOMA ASSOCIATED WITH SPONTANEOUS HYPOGLYCEMIA*

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ABSTRACT

Using the glucose uptake of the isolated rat hemidiaphragm or the rat epididymal fat pad as an assay procedure, a glucose-uptake stimulator was extracted and concentrated from a large retroperitoneal fibrosarcoma removed from a patient with spontaneous hypoglycemia. Fractionation revealed the coexistence of an *in vitro* glucose-uptake inhibitor in the tumor. The glucose-uptake stimulating activity in a 22 per cent ethanol precipitate was destroyed by heating, but it was relatively heat-stable when in the form of the more concentrated zinc acetate precipitate. The stimulating activity was nondialyzable. The material which inhibited glucose uptake appeared to be heat-stable and dialyzable.

INTRODUCTION

OF THE several attempts to demonstrate insulin-like activity in tumors of apparently nonpancreatic origin removed from patients exhibiting spontaneous hypoglycemia (1–5), only that of August and Haitt has been successful (6). Their procedure, carried out by Dr. D. B. Martin, consisted of making a physiologic saline homogenate of the tumor and testing for the insulin activity by measuring the glucose uptake of the rat hemidiaphragm. They reported that the tumor contained as much as 0.5 unit of insulin activity per gram of tissue.

In March 1959, a patient was admitted to this Medical Center exhibiting severe hypoglycemic episodes. The hypoglycemia was completely alleviated by surgical removal of a massive retroperitoneal fibrosarcoma. It was possible to demonstrate markedly elevated levels of insulin-like activity in the patient's serum before removal of the tumor, with a return to normal serum levels after the operation (7). This observation suggested that a

Received November 2, 1960.

* This work was supported by Research Grant #A-1535 from the Institute of Arthritis and Metabolic Diseases, National Institutes of Health.

† Recipient of a Medical Student Summer Fellowship of the National Institutes of Health.

possible explanation of the hypoglycemic episodes was the release from the tumor to the systemic circulation of a substance capable of stimulating glucose utilization in the peripheral tissues, a view that would be strengthened by a demonstration of a glucose-uptake stimulator in tumor extracts. This report presents the demonstration of a glucose-uptake stimulator in extracts of this retroperitoneal fibrosarcoma.

METHODS

Preservation of the tissue

A retroperitoneal fibrosarcoma weighing 1570 Gm. was resected from a 39-year-old white female who had had repeated episodes of severe, spontaneous hypoglycemia. (For the case report, see Whitney and Heller (7).) Half of the tumor was cut into slices approximately 3 cm. wide. Within forty-five minutes after removal, each slice was frozen between blocks of dry ice. It was apparent on gross observation that the tumor contained large necrotic areas, and an attempt was made to separate the necrotic from the non-necrotic parts before freezing. These different parts were wrapped and stored separately. After the first freezing, the tissue was kept at −18° C. Small pieces were shaved off the block of frozen tumor for fractionation and assay. The non-necrotic tissue was used in the first three assays to be described, and the necrotic tissue in the last experiment.

Assay for glucose-uptake stimulating activity

Various extracts or fractions of the tumor were assayed by testing their effect on the glucose uptake of either the rat hemidiaphragm or the epididymal fat pad. Because the assays with hemidiaphragms were carried out only in the preliminary stages of working out a fractionation procedure and were qualitatively similar to the assays with fat pads, only the results of assays with fat pads are reported here. An increase in the glucose uptake of the fat pads or hemidiaphragms incubated in the tumor extract as compared to the glucose uptake in buffer was interpreted as representing insulin-like activity. No attempt was made to quantitate the insulin-like activity in terms of units of insulin per gram of tissue for two reasons: the variability among techniques and laboratories is very large (8), and there is as yet no ground for believing that the glucose-uptake stimulating material described here is insulin.

For the assay of glucose-uptake stimulating activity, intact fat pads were used from rats which had received no food the previous night. The fad pads were incubated in 1 ml. of the tumor fraction in buffer, which had a glucose concentration of 2.5 mg. per ml. The glucose uptake of the fat pads in buffer alone was measured as a control basal uptake in each assay. The incubation period was three hours at 37° C. in a gas phase of 95 per cent O_2–5 per cent CO_2. Glucose was determined by the method of Somogyi (9).

The nitrogen content of some of the fractions was determined by a semi-micro Kjeldahl method using boiling chips impregnated with selenium oxychloride during the digestion of the sample with concentrated sulfuric acid. The nitrogen content of the samples was used to calculate the specific activity of each fraction in terms of the change in glucose uptake over that in the control buffer per milligram of nitrogen.

Tumor fractionation procedure

All manipulations of the tumor were performed at 3–5° C. To simplify preparations of the tumor in the first assays carried out on buffer extracts, all tumor extracts were made

in Krebs Ringer bicarbonate buffer pH 7.4 containing 2.5 mg. per ml. of glucose (hereafter referred to as buffer). More than 25 separate fractionations and assays were carried out in an attempt to concentrate the glucose-uptake stimulating activity. Only representative fractionations and assays are presented here.

The fractionation procedure which resulted in the highest yields of activity is outlined as follows: A 10-Gm. portion of the fibrosarcoma was homogenized in 30 ml. of buffer. The homogenate was centrifuged at 18,000×g, and the precipitate was discarded. A 7-ml. aliquot of the supernatant was dialyzed against buffer overnight. The remainder of the supernatant was brought to a concentration of 22 per cent ethanol by the slow addition of 95 per cent ethanol over a period of two hours. The precipitate which formed was centrifuged and the supernatant was discarded since it had been determined that this supernatant contained negligible amounts of glucose-uptake stimulating activity. The precipitate was suspended in 28 ml. of buffer and was divided into four 7-ml. aliquots. One aliquot was saved for assay without further treatment. One aliquot was dialyzed against buffer. Two aliquots were heated in a boiling water bath for thirty minutes; one of these aliquots was saved for assay and the other was dialyzed against buffer overnight.

In a separate series of fractionations the stimulating activity of the 22 per cent ethanol precipitate was further concentrated by subjecting the precipitate to extraction with buffer, centrifuging, and adding approximately 0.2 ml. of 10 per cent zinc acetate per 10 ml. of the supernatant. The resulting precipitate was centrifuged and saved. This supernatant was discarded. The zinc acetate precipitate was tested, as was the 22 per cent ethanol precipitate: *a*) without further treatment, *b*) after dialysis against buffer, *c*) after heating, and *d*) after heating followed by dialysis.

The precipitates were assayed for glucose-uptake stimulating activity after suspending them in buffer by the use of a Potter-Elvejhem homogenizer.

RESULTS

Preliminary assays of simple buffer extracts of the tumor yielded variable results. In 1 highly concentrated extract, inhibition of glucose uptake was observed. More dilute extracts either caused no stimulation of glucose uptake or a slight (but statistically significant) increase, with both hemidiaphragms and fat pads. The application of two published procedures (10, 11) for the extraction of insulin from pancreas resulted in some concentration of the glucose-uptake stimulating activity, but the yields were low enough to warrant an attempt to devise the separate procedure outlined in the preceding section.

The data are presented in Table 1. Experiment A is the summary of 2 separate fractionations and assays. In these fractionations the "non-necrotic" portions of the tumor were used. Experiment B is the summary of 3 fractionations of the "necrotic" portions of the tumor. The necrotic portions were used because the supply of non-necrotic tissue was nearly exhausted. In both series, the supernatant of the initial buffer extract caused a significant increase in glucose uptake. Comparison of the specific activities indicates that the activity of the necrotic portion of the tumor was only half that of the non-necrotic portion.

TABLE 1. GLUCOSE UPTAKES (RAT EPIDIDYMAL FAT PADS) FROM FRACTIONS OF RETROPERITONEAL FIBROSARCOMA

Group No.	Incubation medium	Glucose uptake (mg./Gm./3 hrs.)	P values*	Change (%)	Specific activity†
		Experiment A			
1	Buffer	4.18 ± .73 [10]**			
2	Homogenate‡	9.46 ± .81 [5]	< .001 (2-1)	+226	+ 1.19
3	22% ppt. untreated	7.66 ± .52 [10]	< .01 (3-1)	+183	+ 2.81
4	22% ppt. dialyzed	11.22 ± .67 [10]	< .01 (4-3)	+268	+ 6.58
5	22% ppt. heated	1.96 ± .40 [10]	< .001 (5-3) < .001 (5-4) < .02 (5-1)	− 47	− 2.14
6	22% ppt. heated & dialyzed	3.63 ± .40 [10]	< .05 (5-6)	− 9	− 0.63
		Experiment B			
1	Buffer	1.36 ± .14 [15]			
2	Homogenate‡	3.11 ± .20 [10]	< .001 (2-1)	+228	+ 0.52
3	22% ppt. untreated	2.97 ± .20 [15]	< .001 (3-1)	+218	+ 1.77
4	22% ppt. dialyzed	3.18 ± .22 [15]	< .001 (4-1) n.s. (3-4)	+233	+ 2.25
5	22% ppt. heated	1.09 ± .17 [15]	n.s. (5-1)	− 8	− 0.33
6	22% ppt. heated & dialyzed	1.16 ± .14 [15]	n.s. (6-1)	− 9	− 0.24
7	ZnAc ppt. untreated	3.05 ± .21 [15]	< .001 (7-1)	+224	+14.10
8	ZnAc ppt. dialyzed	2.95 ± .22 [15]	n.s. (8-7)	+216	+14.40
9	ZnAc ppt. heated	2.24 ± .26 [15]	< .02 (9-7)	+165	+ 7.33
10	ZnAc ppt. heated & dialyzed	2.66 ± .17 [15]	< .02 (10-9) n.s. (10-7)	+195	+ 9.30

* P compares groups shown in parentheses; n.s. = not significant. P is derived from t where

$$t = \frac{m_1 - m_2}{\sqrt{\left(\frac{\Sigma d_1^2 + \Sigma d_2^2}{n_1 + n_2 - 2}\right)(1/n_1 + 1/n_2)}}$$

† The difference in glucose uptake in the fractions from that in buffer per mg. of nitrogen.

** Mean ± standard error of the mean. The number in brackets denotes the number of fat pads in each group.

‡ The buffer homogenate of the tissue was centrifuged at 18,000 × g and only the supernatant was used in the incubation.

In the 22 per cent ethanol precipitate there was a two- to threefold increase in specific activity. Dialysis of the 22 per cent precipitate resulted in a further increase in specific activity, particularly in the non-necrotic portions of the tumor (compare Groups 4, Experiments A and B). Heating the 22 per cent precipitate not only destroyed the stimulating activity from both portions of the tumor, but unmasked the presence of a glucose-uptake inhibitor, at least in the non-necrotic portion. Dialysis of the heat-treated sample removed the inhibitor (compare Groups 6 and 5, Experiment A). There was no significant difference between the glucose uptake in the heated and dialyzed precipitate as compared to that in buffer alone.

In Experiment B, a further concentration of the stimulator was achieved by precipitation of the 22 per cent ethanol-precipitate-extract by the addition of zinc acetate. Dialysis did not enhance the activity. This fraction was less sensitive to heat than was the 22 per cent precipitate, although heating reduced the stimulating activity significantly and the specific activity by half (compare Groups 9 and 7, Experiment B). Dialysis of the heated zinc acetate fraction caused a small but significant increase in the stimulator activity and in the specific activity (compare Groups 10 and 9, Experiment B). There was no statistically significant difference between the heated and dialyzed zinc acetate fraction and the untreated fraction.

DISCUSSION

It is clear from the data presented that this retroperitoneal fibrosarcoma removed from a patient with spontaneous hypoglycemia contained large amounts of a material which increased the glucose uptake of rat epididymal fat pads *in vitro*. Other experiments, not included here, have shown that the glucose uptake of hemidiaphragms is also stimulated. Since it was possible to demonstrate increased insulin-like activity in this patient's serum during hypoglycemic episodes and a return to normal levels following removal of the tumor, it is reasonable to assume that the tumor released a material into the systemic circulation which accounted for the hypoglycemia. For the sake of this discussion and to avoid any implication as to the composition of the glucose-uptake stimulating activity, the material will be referred to as the stimulator.

This stimulator has not been well characterized. However, several of its properties are apparent from these experiments. It withstands freezing since it can be extracted from frozen tissue, and it is non-dialyzable. The thermal lability of the stimulator is difficult to assess. Heating destroyed the stimulating activity of the 22 per cent ethanol precipitate in all experiments but did not completely destroy the activity in the zinc acetate precipitate. Several explanations for this disparity could be postulated. Two of the more likely possibilities are: 1) that the more crude 22 per cent

ethanol precipitate contained materials which destroyed the stimulator at high temperatures, whereas these materials had been partially removed by precipitating the stimulator with zinc acetate, or 2) that by being precipitated and possibly bound to zinc, the stimulator was less susceptible to denaturation by heating.

Of perhaps equal importance was the demonstration of the co-existence in this tumor of material which inhibits the uptake of glucose *in vitro* (hereafter referred to as the inhibitor). This inhibitor is heat-stable and dialyzable. The presence of the inhibitor made the concentration of the stimulator more difficult. The consistent presence of an inhibitor in other reported tumors associated with spontaneous hypoglycemia might account in part for the failure of other investigators to demonstrate stimulator activity in extracts of these tumors (1–5). It should be noted that the specific activities of both the inhibitor and stimulator were much lower in the necrotic portion of the tumor. The difference in the amounts of inhibitor removed by dialysis in Experiment A, as compared to B, might have been due to a difference in the ratio of inhibitor to stimulator, dependent upon the relative amount of necrosis. August and Hiatt (6) noted differences in insulin-like activity in histologically different portions of their tumor.

Attempts have been made to separate the factors involved in the stimulation and inhibition of glucose uptake, both chromatographically and electrophoretically. So far, the identification of discrete components associated with biologic activity has failed.

A review of the literature shows that the majority of the tumors found in patients exhibiting spontaneous hypoglycemia were very large; yet hypoglycemic symptoms appeared only a short time before surgery or death. In at least 1 case the tumor was demonstrated a year before hypoglycemia appeared (6). One could postulate that the hypoglycemic activity is present in these tumors from the time they begin to grow, but the co-existing inhibitor of glucose uptake is present in sufficient quantity to prevent any notable decrease in the concentration of blood sugar. As the tumor increases in size it is possible that either the production or release of the stimulator is increased out of proportion to the inhibitor, resulting in the onset of hypoglycemic symptoms. The answers to these questions must await the discovery and study of another case of this interesting and apparently rare syndrome.

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KIDNEY FUNCTION IN VARIOUS THYROID STATES*

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ABSTRACT

Twenty-two patients were studied by means of discrete renal function analysis—7 with hyperthyroidism, 3 with hypothyroidism, and the remainder with other clinical states—before and after treatment with triiodothyronine or thyroxine. Following definitive therapy of the hyperthyroid state, there was a greater depression in renal hemodynamic functions (GFR and RPF) than in renal tubular activity (TmPAH), probably due to reduction in cardiovascular dynamics. Following short-term treatment of the hypothyroid state with thyroxine or triiodothyronine, there was a greater increase in TmPAH than in GFR and RPF, suggesting that this is the primary effect of active thyroid agents on the kidney.

INTRODUCTION

A CLEAR delineation of the alterations in renal physiology in thyroid dysfunction is of interest not only in the consideration of replacement therapy but in view of the increasing use of iatrogenic hypothyroidism as indirect therapy for severe cardiac and respiratory conditions. Changes in glomerular filtration rate (GFR) renal plasma flow (RPF) and tubular excretory capacity (TmPAH) have been observed in hypothyroidism and in hyperthyroidism (1–7). To develop an understanding of the dynamics in these parameters of renal function, we studied a group of patients both before and after definitive therapy in states of hyperthyroidism, euthyroidism and hypothyroidism. The therapeutic agents included radioiodine (I^{131}), thyroxine and triiodothyronine.[1]

MATERIALS AND METHODS

Twenty-two patients were studied by means of discrete renal function analysis for the determination of glomerular filtration rate (inulin clearance), renal plasma flow (para-aminohippurate clearance), and the tubular excretory capacity for para-amino-

Received November 18, 1960.

* Supported in part by a grant from the Houston Heart Association.

[1] Triiodothyronine supplied by courtesy Smith, Kline & French Laboratories, Philadelphia, Pennsylvania, and Hoffman-LaRoche, Inc., Summit, New Jersey.

hippurate (TmPAH). The techniques and methods of analysis have been previously described (8).

Seven of the patients were hyperthyroid and were studied before and after definitive therapy. In 2 of these, the follow-up study was performed eight weeks after thyroidectomy. In the remaining 5, the follow-up study was performed three weeks after therapeutic doses of I^{131}. The clinical status of these patients is briefly outlined in Table 1.

Thirteen of the patients were studied before and after the oral administration of

TABLE 1. DISCRETE RENAL FUNCTION STUDIES IN 7 HYPERTHYROID PATIENTS BEFORE AND AFTER TREATMENT

Patient		Post-treatment interval (wks.)	BMR (%)		Thyroidal 24-hour I^{131} uptake (% dose)	
No.	Age, sex & race		B*	A*	B	A
		Thyroidectomy				
1	41, MW	8	+22	−18	47	0.8
2	39, MN	8	+46	+12	59	4
		Treated with Radioactive Iodine				
3	23, MW	3	+26	−11	42	18
4	30, MW	3	+25	−19	47	4
5	32, MW	3	+15	−17	36	24
6**	30, MW	3	−13	−40	36	10
7**	32, MW	3	+39	− 9	61	6

Patient	GFR† (ml./min.)		RPF† (ml./min.)		TmPAH† (mg./min.)		F Fraction†		GFR/TmPAH†		RPF/Tm†	
	B	A	B	A	B	A	B	A	B	A	Control	Drug
					Thyroidectomy							
1	138	35	1638	149	115	94	0.08	0.23	1.2	0.37	14.2	1.6
2	151	106	767	547	127	120	0.20	0.19	1.2	0.88	6.0	4.6
					Treated with Radioactive Iodine							
3	139	85	1195	927	118	96	0.12	0.09	1.2	0.89	10.1	9.7
4	95	74	659	533	93	70	0.14	0.14	1.02	1.06	7.1	7.6
5	126	103	968	564	87	85	0.13	0.18	1.4	1.2	11.1	6.6
6**	111	74	805	536	105	89	0.14	0.14	1.06	0.83	7.7	6.0
7**	217	111	1596	772	134	88	0.14	0.14	1.62	1.3	11.9	8.8

* B = before treatment; A = after treatment.
** Extraction of PAHA by renal-vein catheterization:

Pt.	*B*	*A*
6	92%	78%
7	92%	66%

† For key, see footnotes to Table 2.

triiodothyronine. The hormone was given in a single dose each day, beginning the day after control renal function studies had been made. The doses were 25, 50, 75 and 100 μg. on the successive days. Two hours after the last dose, renal function was again studied. In this group were 3 normal subjects, 3 hypothyroid patients, 3 patients with glomerulonephritis, and 4 patients with panhypopituitarism. Descriptive clinical information is presented in Table 2.

Two normal subjects were studied before and after the oral administration of thyroxine. Thyroxine was given in a dosage of 0.6 mg. (one dose) daily for four consecutive days. Renal function studies were repeated on the second and fifth days. Certain clinical data are presented in Table 3.

RESULTS

Hyperthyroidism (Table 1)

Before treatment the GFR, RPF and TmPAH were all normal or greater than normal. Following treatment by thyroidectomy or therapeutic doses of I^{131} (varying from 3 to 10 μc.) there was a reduction in all functions studied. The filtration fraction was variable in its response but the GFR/TmPAH and RPF/Tm ratios declined, indicating that the tubular excretory capacity for para-aminohippurate was reduced less than the filtration rate and renal plasma flow. In Case 1 there was a marked decrease in all functions studied. This was correlated with the marked decrease in I^{131} uptake (0.8 per cent) at the time of the study, indicating severe hypothyroidism.

Two patients (Nos. 6 and 7) were studied three weeks after receiving therapeutic dosages of I^{131} by means of renal vein catheterization for the extraction of para-aminohippuric acid (PAHA) by the kidney. There was a marked reduction in E_{PAH} (from 92 per cent before treatment in both cases, to 78 per cent in one and 66 per cent in the other). These 2 patients were the only ones who demonstrated a clinically significant renal abnormality, namely, oliguria for a period of three to five days, in association with albumin (1–2 plus) and cellular casts in the urine. This syndrome appeared in the fourth week following therapy. Thus, there are two alternative explanations for the marked decrease in the extraction of PAHA: 1) decreased activity of the tubular transport mechanism for PAHA, resulting from a reduction in thyroid activity, or 2) a toxic effect of a therapeutic dose of radioactive iodine on the renal tubules.

Subjects receiving triiodothyronine (Table 2)

Included in this group were 3 patients with hypothyroidism (Nos. 11, 12 and 13) whose discrete renal functions were all subnormal. Following four days of therapy with triiodothyronine there was very little change in GFR or RPF, except in Patient No. 12 in whom a significant increase in RPF occurred. Incidentally, this patient also exhibited the greatest degree of hypothyroidism, as determined clinically and by the low 24-hour thyroidal uptake of I^{131} (2 per cent). In all 3 patients there was a significant increase in TmPAH, and the GFR/Tm and RPF/Tm ratio was decreased, indicating a disproportionately greater effect on tubular activity compared to filtration rate and renal plasma flow, except in Patient No. 12.

In the 3 normal subjects and the 3 patients with glomerulonephritis, no significant changes occurred following administration of triiodothyronine.

In the 4 patients with panhypopituitarism there was no significant change in GFR or RPF. However, in 2 of these (Nos. 18 and 20) there was

TABLE 2. DISCRETE RENAL FUNCTION STUDIES FOLLOWING ADMINISTRATION OF TRIIODOTHYRONINE*

Subject		BMR (%)	Thyroidal 24-hour I^{131} uptake (% dose)	GFR† (ml./min.)		RPF† (ml./min.)		RPF/Tm†		TmPAH† (mg./min.)		FF†		GFR/TmPAH	
No.	Age, sex & race			C†	D†	C	D	C	D	C	D	C	D	C	D
Group I—Normal Subjects															
8	32, MW	−10	22	95	120	852	753	10.8	10.3	79	73	0.11	0.16	1.2	1.6
9	37, MW	+ 2	26	115	115	808	858	10.2	11.3	79	76	0.14	0.13	1.5	1.5
10	30, MW	+ 1	22	123	121	649	679	5.3	5.7	122	120	0.19	0.18	1.0	1.0
Group II—Hypothyroidism															
11	65, FW	−10	4	60	71	231	212	3.0	2.1	77	101	0.26	0.33	0.78	0.70
12	32, MW	−29	2	54	62	328	607	5.0	6.8	66	89	0.16	0.10	0.82	0.70
13	62, FW	−10	4	83	82	553	610	10.4	7.6	53	80	0.15	0.13	1.6	1.03
Group III—Glomerulonephritis															
14	38, MN	− 9	21	134	132	695	799	5.9	6.4	118	125	0.19	0.17	1.1	1.06
15	35, MN	+ 2	18	50	56	500	404	6.0	5.0	84	81	0.10	0.14	0.60	0.69
16	34, MN	+ 1	24	16	18	138	94	10.6	6.7	13	14	0.12	0.19	1.2	1.3
Group IV—Hypopituitarism															
17	52, MW	−31	9	77	80	521	565	6.1	6.5	85	87	0.15	0.14	0.91	0.92
18	35, MW	−28	6	82	87	784	781	8.7	6.6	90	119	0.10	0.11	0.91	0.73
19	59, MN	−18	8	70	76	574	514	8.7	7.6	66	68	0.12	0.15	1.06	1.1
20	48, FW	−30	7	89	86	556	604	13.6	8.4	41	72	0.16	0.14	2.2	1.2

* Triiodothyronine administered orally: 25 μg. on first day, 50 μg. on second day, 75 μg. on third day, and 100 μg. on fourth day. "Drug" study performed on fourth day, two hours after the 100-μg. dose.
C = Control; D = Drug.
GFR = Glomerular filtration rate.
RPF = Renal plasma flow.
Tm = Tubular maximum excretory capacity; PAH = for para-amino hippurate.
FF = Filtration fraction.

an increase in TmPAH with a decrease in the GFR/Tm and RPF/Tm ratio similar to that seen in the hypothyroid group. These 2 patients presented the greatest evidence of secondary hypothyroidism as determined clinically and by the low 24-hour thyroidal uptake of I^{131} (6 and 7 per cent, respectively).

Subjects receiving thyroxine (Table 3)

In the 2 normal subjects (Nos. 21 and 22) who received thyroxine orally, slight increases in GFR, RPF and TmPAH were observed twenty-four hours after the first dose (0.6 mg.). After ninety-six hours of therapy (0.6 mg. daily) there was no further increase in GFR but the RPF continued to rise. In both of the patients, TmPAH was increased after the first dose, and in one patient it continued to rise to the very high level of 148 mg. per minute. These changes were reflected further in a declining GFR/Tm ratio, especially after ninety-six hours of therapy, indicating greater augmentation of tubular capacity for PAHA than of filtration rate. However, there was an increase in the RPF/Tm ratio, indicating greater augmentation of renal plasma flow than of tubular function.

Although the changes following administration of thyroxine were greater

than those following administration of triiodothyronine, it should be emphasized that the total dosage of thyroxine on a weight-of-drug basis was ten times greater than that of triiodothyronine.

DISCUSSION

It has been noted (1–7) that renal function tends to be depressed in patients with primary hypothyroidism as well as in those with hypothyroidism secondary to hypopituitarism, with a trend toward normal function following thyroid therapy. A general increase in renal functional activity has been reported in hyperthyroidism (1, 2), with variable changes following thyroidectomy. Others (9, 10) have noted that administration of thyroxine to normal dogs results in an increase in RPF, GFR and the tubular maximal excretory capacity for glucose. Our data show that, in the

TABLE 3. DISCRETE RENAL FUNCTION STUDIES IN 2 NORMAL SUBJECTS AFTER ADMINISTRATION OF THYROXINE*

	Patient #21	Patient #22
Age (yrs.)	38	33
Sex	F	M
Race	Negro	Negro
Basal metabolic rate (%):		
Control	+ 3	−24
Drug (0.6 mg.)	+ 6	− 5
Drug (2.4 mg.)	+24	+ 5
Glomerular filtration rate (ml./min.):		
Control	83	98
Drug (0.6 mg.)	98	113
Drug (2.4 mg.)	81	115
Renal plasma flow (ml./min.)		
Control	531	437
Drug (0.6 mg.)	559	745
Drug (2.4 mg.)	670	1166
Tubular maximal excretory capacity/PAH (mg./min.):		
Control	110	111
Drug (0.6 mg.)	130	124
Drug (2.4 mg.)	130	148
Filtration fraction:		
Control	0.16	0.22
Drug (0.6 mg.)	0.17	0.15
Drug (2.4 mg.)	0.12	0.10
GFR/TmPAH:		
Control	0.75	0.88
Drug (0.6 mg.)	0.74	0.91
Drug (2.4 mg.)	0.62	0.78
Renal plasma flow/Tm:		
Control	4.8	3.9
Drug (0.6 mg.)	4.3	6.0
Drug (2.4 mg.)	5.1	7.9

* Drug studies were performed twenty-four hours following the administration of 0.6 mg. of thyroxine orally, and on the fourth day of administration of 0.6 mg. thyroxine daily.

hyperthyroid state, cardiovascular dynamics are augmented sufficiently to increase GFR and RPF out of proportion to the changes in kidney tubular function.

The results of this study indicate that triiodothyronine, in the dosage used, exerts its effect principally under conditions of thyroid hormone deficiency, since none of the euthyroid patients demonstrated a significant alteration in renal function. The data on the patients with primary and secondary hypothyroidism indicate that metabolic stimulation at the tissue level (rather than an alteration in renal hemodynamics) represents the primary renal action of triiodothyronine. This is demonstrated by a decrease in the GFR/TmPAH ratio, implying a greater change in renal tubular capacity than in filtration rate.

Thus, one may postulate that the hyperthyroid state is accompanied by an increased cardiac output which is reflected in an increased GFR and RPF out of proportion to the renal tubular capacity. In the hypothyroid state, metabolism is depressed. Following substitution therapy, the renal tubular capacity for PAHA is augmented before the general circulatory increases can be expressed through an augmented GFR or RPF. This suggests that there is a primary effect of triiodothyronine on the kidney, and that changes in GFR and RPF are renal effects which are merely secondary to increased cardiovascular dynamics.

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EFFECT OF THYROTROPIN UPON RELEASE OF IODIDE FROM HUMAN AND RAT THYROID*†

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ABSTRACT

The early effects of thyrotropin (TSH) upon urinary and thyroidal iodide were studied in man and in the rat. *Human subjects.* (*a*) TSH given intramuscularly to euthyroid and hyperthyroid persons whose thyroidal iodine stores had been labeled with I^{131} produced within two to four hours an increase in the urinary excretion of iodide-I^{131} which was maximal in twelve to twenty-four hours. Myxedematous subjects failed to respond. (*b*) When organic binding was prevented (by methimazole) and iodide-trapping was partially impaired (by thiocyanate), the injection of TSH led, in most cases, to a prompt decrease in glandular radioiodide content. *Rats.* (*a*) The urinary excretion of I^{131} increased significantly within two and a half hours after administration of TSH to intact rats given I^{131} several days earlier; in thyroidectomized rats there was a smaller, but distinct, increment in urinary I^{131}. (*b*) When organic binding of injected I^{131} was blocked (by propylthiouracil) and thyroidal iodine stores were unlabeled, the glandular concentration of I^{131} was distinctly lower fifteen to one hundred and twenty minutes after intravenous TSH than it had been before injection. (*c*) The percentage of iodide-I^{131} in glands in which the organic iodine stores had been labeled and in which organic binding was not blocked, rose promptly after injection of TSH. The results are compatible with the hypothesis that in these two species the administration of TSH leads to an increase in the intrathyroidal concentration of iodide derived from deiodination reactions, and the iodide so formed may be released from the gland.

STUDIES in the dog of thyroid venous blood obtained before and after the administration of thyrotropin (TSH) have indicated that TSH induces the prompt release from the thyroid not only of hormonal iodine but also of inorganic iodide (1, 2). Evidence has been presented suggesting that the iodide so released arises within the gland, from the deiodination of

Received October 17, 1960.

* Presented at the Forty-second Meeting of The Endocrine Society, Miami Beach, Florida, June 9–11, 1960.

† This investigation was supported by a research grant (A-2585) from the Institute of Arthritis and Metabolic Diseases, Public Health Service.

organic iodine compounds (1, 3). It seemed of interest to determine whether TSH might affect the thyroidal release of iodide in man and in the rat. The problem was approached by studying the effect of TSH administration upon 1) the urinary excretion of radioiodine after glandular iodine stores had been labeled by I^{131}, and 2) the concentration of radioiodide within the thyroid under varying experimental conditions.

METHODS

Urinary excretion of radioiodine

Human subjects. Fourteen adult persons, hospitalized for a variety of disease states, were studied; the group included 10 euthyroid, 2 thyrotoxic, and 2 myxedematous patients. A tracer dose of I^{131} (50–100 μc. of Oriodide (Squibb), oral therapeutic solution) was given orally to each subject. In all but 3 cases, the oral administration of potassium perchlorate (100 mg. every six hours) was begun two to four days later and continued throughout the remainder of the study period, in order to minimize further active accumulation of iodide by the thyroid. A single intramuscular injection of TSH (10 units, Armour Thytropar) was given four to six days after administration of I^{131}. Urine was collected in consecutive periods, beginning forty-eight to seventy-two hours after I^{131} and continuing for five to eight days after injection of TSH. The collection periods were generally six to twelve hours in length, but in several cases a three-hour interval was feasible, and in 2 subjects bearing indwelling urethral catheters the urine was collected every two hours. Toward the end of the study, the collection period was extended to twenty-four hours. The volume of urine collected in each period was measured and the radioactivity of a suitable aliquot determined in a well-crystal scintillation detector.

Rats. The urinary excretion of I^{131} before and after the administration of TSH was also studied in rats which had been given I^{131} (1–3 μc. intraperitoneally) two or three days earlier. Male rats weighing approximately 100 Gm. each were given a low-iodine (Remington) diet[1] and water until eighteen hours before the collection of urine was begun, when a 1 per cent solution of sodium thiocyanate was substituted for the drinking water. At the end of this period, the rats were placed in individual metabolism cages and diuresis was initiated and maintained by giving each animal 3 ml. of water every ninety minutes by stomach tube. The urinary excretion of I^{131} during a 2½-hour period before intraperitoneal injection of TSH (0.5–1.0 unit in physiologic saline) was compared with that in an equal period after injection. Animals receiving injections of saline alone served as controls. Similar experiments were performed using rats that had been surgically thyroidectomized a few days to several weeks earlier.

Radioiodide content of the thyroid

Human subjects. The glandular radioiodide content was studied before and after the administration of TSH to subjects given repeated doses of methimazole in order to block the organic binding of iodine in the thyroid. In 8 cases the procedure was as follows: a dose of 30 mg. of methimazole was given orally and two hours later 50 μc. of I^{131} was given by mouth. Two hours after the tracer dose of I^{131} had been ingested, when it seemed likely that equilibrium between thyroid iodide and plasma iodide had been attained, the radioactivity in the thyroid region was estimated by external counting using a collimated scintillation detector. Correction of the neck count to allow for extrathy-

[1] Iodine-deficient test diet, General Biochemicals, Inc., Chagrin Falls, Ohio.

roidal radioactivity was made by subtracting the body background count (thigh) obtained simultaneously by the use of another counter. These measurements were repeated at intervals of fifteen to sixty minutes, and when a sufficient number of observations had been made to define the slope of the curve relating glandular radioactivity with time, a single intramuscular injection of TSH (10 units) was [illegible]d measurements continued for several hours more. Administration of methimaz[illegible]20–30 mg.) was repeated every four to six hours during the study. In another group [illegible]f 8 cases, the same procedure was followed except that a single dose of sodium thiocyanate (100 mg.) was given together with the initial dose of methimazole.

Rats. The early effect of TSH upon thyroidal iodide concentration was studied when organic binding of iodine was prevented and also when it was permitted. In one group of experiments, rats which had been maintained with a Purina diet for one week were used. Each animal was given 100 mg. of propylthiouracil subcutaneously. Thirty minutes later 0.5 μc. of I^{131} was injected intraperitoneally. One hour after administration of the I^{131} tracer the animal was anesthetized with ether, the right lobe of the thyroid was removed and weighed, and TSH (0.1–1.0 unit) was immediately injected intravenously. Either fifteen minutes or two hours later, the left lobe of the gland was removed and weighed. Control animals were subjected to the same procedure except that physiologic saline was injected instead of TSH. The hemithyroids were dissolved in 2*N* sodium hydroxide and radioactivity was determined using a scintillation well-counter.

The effect of TSH on the radioiodide content of the unblocked gland was also studied when the iodine of the thyroid had been previously labeled. Rats which had been fed a low-iodine diet for one week were given I^{131} (5–25 μc.). Beginning twenty-four hours later they were given a single injection of 5 μg. of *l*-thyroxine daily for two days, to minimize release of thyrotropin from the pituitary. Eighteen hours after the last injection, each animal was anesthetized (ether) and the right thyroid lobe removed; immediately after this, TSH (1 unit) was given intravenously and the left lobe was later removed. In some experiments the interval between removal of the right and left lobes was fifteen minutes, whereas in others it ranged from thirty minutes to two hours. The thyroid lobes, which had been frozen promptly after excision, were individually applied to separate strips of Whatman 3 MM filter paper previously dampened in barbital buffer (pH 8.6, ionic strength 0.075) and crushed, as in the technique described by Pitt-Rivers and co-workers (4). Inorganic iodide was separated from the remainder of the labeled constituents by electrophoresis on paper (three hours, 200 volts) and the fraction of the total I^{131} present as inorganic iodide was determined by planimetry of the automatically recorded scan pattern of the electrophoretogram (5). Similar experiments were performed using rats in which the glandular iodine stores were poorly labeled relative to the circulating iodide. The right lobe of the gland was removed half an hour after the I^{131} tracer had been given, and the left lobe was removed half an hour after injection of TSH.

RESULTS

Urinary excretion of radioiodine

Injection of TSH under the described conditions led to a prompt increase in urinary I^{131} in both man and the intact rat.

Human subjects. The results of a typical study in a hyperthyroid human subject are shown in Figure 1. Administration of TSH to perchlorate-treated euthyroid or hyperthyroid subjects who had several days earlier

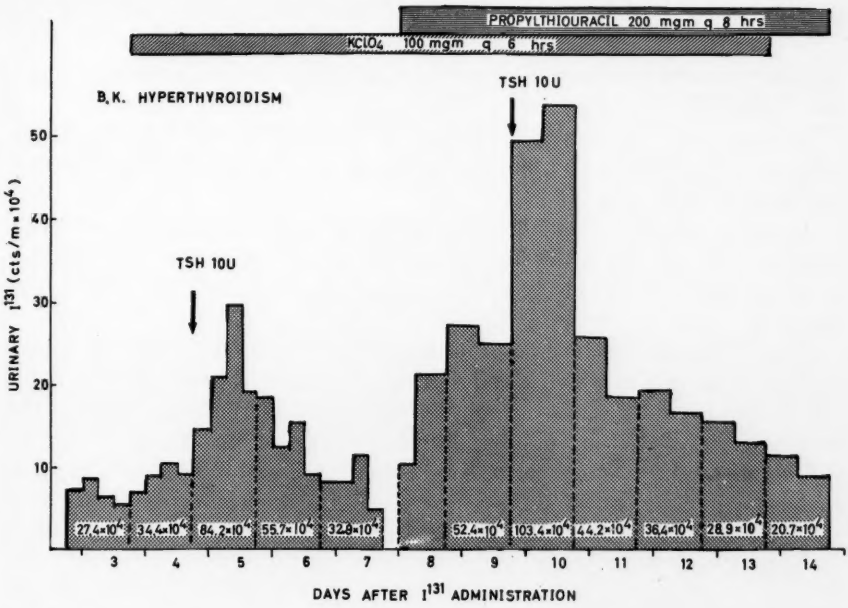

FIG. 1. Effect of TSH upon urinary excretion of I^{131} in a perchlorate-treated hyperthyroid subject. Each collection period (6 or 12 hours) is represented by a bar, the height of which indicates the quantity of radioactivity excreted in each period; numerals in the gray areas refer to the 24-hour urinary excretion of I^{131}.

been given I^{131} produced a two- to sixfold increase in the urinary excretion of I^{131} as compared with the excretion in a comparable period immediately preceding TSH injection. The enhanced urinary excretion of I^{131} was almost always apparent within six hours after administration of TSH, was generally maximal at twelve to twenty-four hours, and persisted for forty-eight hours or more. In the study shown in Figure 1, the response to TSH was also determined after therapy with propylthiouracil had been started; the results were generally similar. The increased urinary excretion of I^{131} associated with the administration of propylthiouracil was probably caused, at least in part, by further inhibition of glandular re-utilization of labeled iodide arising from peripheral degradation of thyroid hormone.

A similar response occurred in the absence of perchlorate (3 cases), but the enhancement of urinary I^{131} excretion consequent to administration of TSH was considerably greater when such euthyroid subjects had been pre-treated with perchlorate (Fig. 2). Administration of perchlorate alone produced an increase in urinary I^{131} excretion of varying magnitude, but it was always less than that subsequently induced by TSH.

Urinary I^{131} in both the pre-TSH and post-TSH periods behaved like iodide, in that it was soluble in trichloroacetic acid and was almost completely removed from acidified urine by an anion-exchange resin (Amberlite IRA-400).

In contrast to the results in euthyroid and hyperthyroid subjects, in the 2 cases of myxedema no appreciable change in urinary I^{131} occurred after injection of TSH (Fig. 3).

In those studies in which urine specimens were collected at intervals shorter than six hours, the increase in urinary I^{131} excretion occurred two to four hours after injection of TSH in 2 cases and three to six hours afterward in 1 case.

Rats. TSH also produced a prompt increase in the rate of urinary excretion of I^{131} in rats in which glandular iodine stores had been labeled (Table 1). Although there was considerable variability in the magnitude of the response to TSH, the animals almost always excreted more I^{131} in the urine during the $2\frac{1}{2}$-hour period after administration of TSH as compared with the period before, and the increments of 50 to 100 per cent were statistically significant ($P < 0.01$). In many experiments the administration

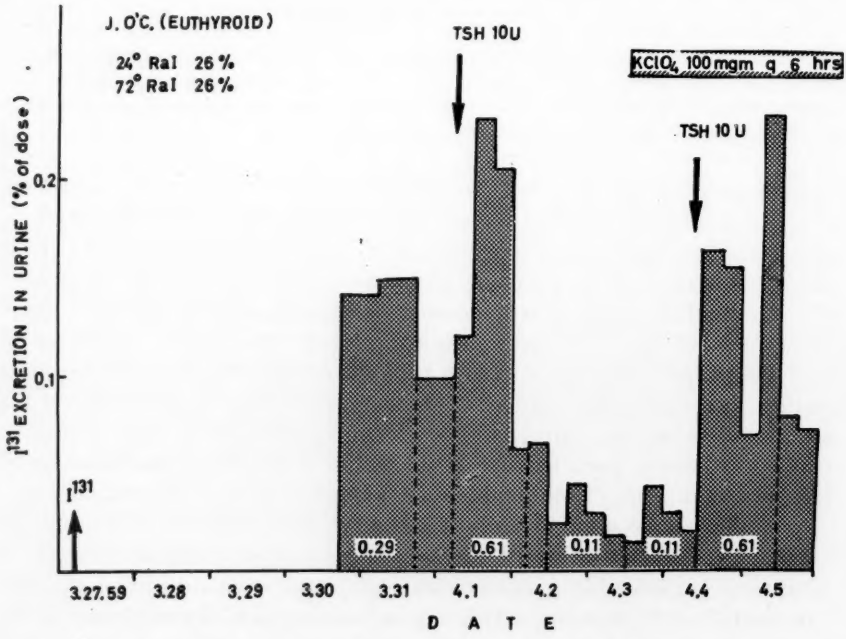

FIG. 2. Enhancement by perchlorate of the increased urinary excretion of I^{131} induced by TSH in a euthyroid subject.

TABLE 1. EFFECT OF TSH ON URINARY RADIOACTIVITY IN RATS GIVEN I^{131}

Experiment	No. of animals	Time after inj. of I^{131} (hrs.)	Dosage of TSH (U.S.P. units)	Urinary I^{131} excretion after TSH or saline (% of pre-injection value)*	
I. Intact rats	19	48	0.5	148 ± 58†	$P < 0.01$
Intact rats	16	48	0	85 ± 45	
II. Intact rats	21	72	0.5	196 ± 105	$P < 0.001$
Intact rats	20	72	0	92 ± 62	
III. Thyroidectomized rats	18	72	0.5–1.0	116 ± 51	$.05 < P < .10$
Thyroidectomized rats	11	72	0	82 ± 30	

* Urine collected for two and a half hours before and after injection.
† Mean ± standard deviation.

of TSH was followed by a reduction in urine flow; this could have been caused by contamination of the TSH preparation with vasopressin (0.04 U.S.P. unit of vasopressin per U.S.P. unit of TSH in one particular lot (U3502) used).[2] In thyroidectomized rats, injection of TSH also led to some increase in urinary I^{131} excretion as compared with the controls; the increase was much less than that observed in intact rats, and was of dubious statistical significance ($P > 0.05$) but it occurred consistently. It is conceivable that the increased urinary excretion of I^{131} induced in the rat by the TSH preparation used may have been partly a renal effect of the

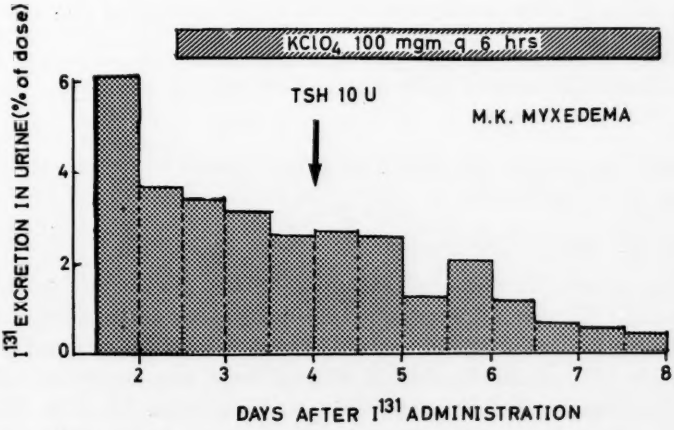

FIG. 3. Urinary excretion of I^{131} in a myxedematous patient before and after administration of TSH.

[2] Dr. J. D. Fisher, Biochemistry Research Department, Armour Pharmaceutical Company kindly provided this information.

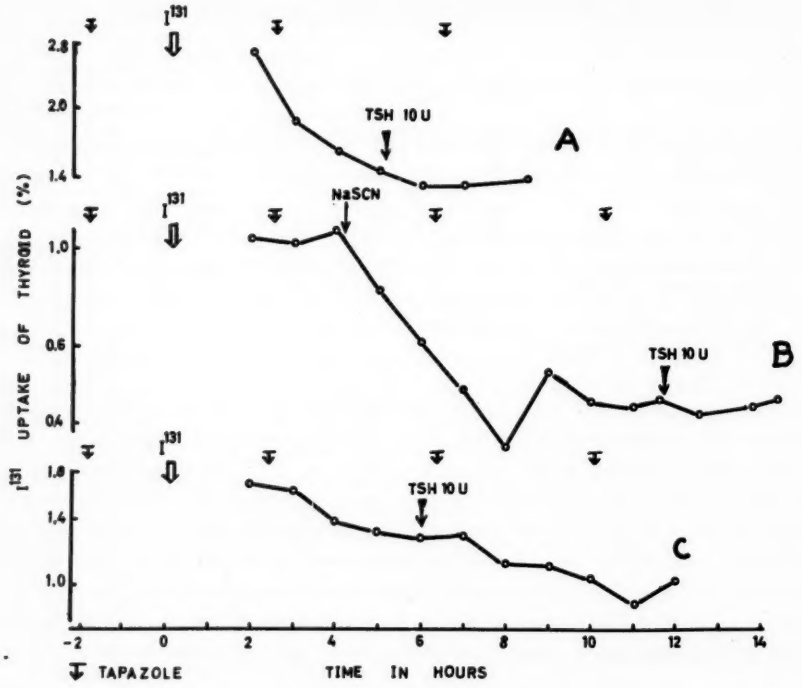

FIG. 4. Effect of TSH upon thyroidal I^{131} in 3 persons pre-treated with methimazole (Tapazole). In contrast to the prompt decrease in glandular radioactivity induced by oral ingestion of sodium thiocyanate (100 mg.), TSH had no apparent effect in A and B, and only a borderline effect in C. Methimazole was given as indicated by short black arrows.

contaminating vasopressin, which has been reported to increase the urinary excretion of I^{131} in mice (6).

Radioiodine content of the thyroid

Human subjects. The administration of TSH to persons pre-treated with methimazole (Tapazole) alone failed, in most cases, to produce a striking early change in glandular radioactivity, as measured by external counting techniques. In 6 of the 8 subjects studied there was no distinct effect of TSH upon the slope of the decay curve of glandular I^{131} (Fig. 4A); in 2 subjects a slight decrease in glandular I^{131} was noted after TSH (Fig. 4C), One of the latter was the only subject to whom TSH was given intravenously (5 ml. of a physiologic sodium chloride solution containing 2 units per ml. injected at a rate of approximately 1 ml. per minute); the thyroidal

I^{131} content decreased appreciably (from 0.72 per cent to 0.57 per cent of the dose) twelve minutes after injection of TSH, but serial observations of glandular radioactivity were discontinued when the patient experienced transient but alarming dyspnea, syncope and vomiting.

The results were somewhat different in the group of 8 cases in which a small quantity of thiocyanate was given together with the initial dose of methimazole in order to decrease but not eliminate the thyroid-plasma iodide-I^{131} concentration gradient. In 5 of these cases a distinct transient decrease in glandular radioactivity was observed soon after injection of TSH (Fig. 5). In 1 case there was an equivocal reduction, and in 2 cases there was no change in the slope of the glandular-I^{131} biologic decay curve.

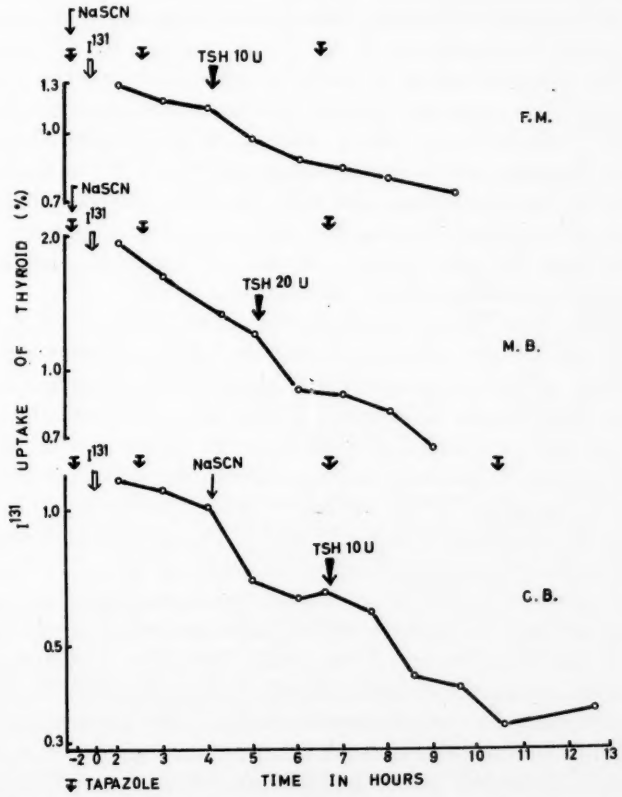

FIG. 5. Effect of TSH upon thyroidal I^{131} in 3 persons pre-treated with methimazole (Tapazole) and 100 mg. of sodium thiocyanate. Additional doses of methimazole were given at times indicated by short arrows. I^{131} given at time zero.

In the cases in which administration of TSH led to decreased glandular content of I^{131}, this response occurred within thirty to forty-five minutes after intramuscular injection; within two hours the rate of glandular loss of iodine returned to the pre-injection level, as judged by the slope of the release curve, which tended to be parallel to the pre-TSH line after this time interval.

Rats. The results of experiments in which the concentration of I^{131} in one lobe of the thyroid (removed just before injection of TSH) was compared with that in the remaining lobe (removed some minutes after injection of TSH) in animals pre-treated with propylthiouracil are shown in Figure 6. The conditions in these experiments were such that organic binding had been virtually completely blocked, glandular I^{131} was present chiefly as inorganic iodide, and equilibrium between thyroidal and plasma iodide-I^{131} had presumably been achieved (7, 8). The concentration of I^{131} in the lobe removed after administration of TSH was appreciably less than that in its fellow at both fifteen and one hundred and twenty minutes after intravenous injection, whereas in the saline-injection controls there was comparatively little difference between the concentrations of I^{131} in the two lobes removed at the corresponding time intervals. The difference between the TSH group and the control group was significant at the 1 per cent level for the 0.3-unit and 1.0-unit dosages; it was of borderline significance ($P = 0.02$) in the experiment in which 0.1 unit was given.

TSH (1 unit) given intravenously to rats in which the thyroid had been pre-labeled with I^{131} given three days earlier led to an increase of approximately 60 per cent in the proportion of glandular I^{131} present as inorganic iodide, as judged by the difference between lobes. In the saline-injection control group there was a moderate decrease (10–25 per cent) in the proportion of I^{131} present as iodide, as calculated from the difference between the percentage of inorganic I^{131} in the lobe removed before injection of TSH and that in the lobe removed after injection. The increase induced by TSH was of approximately the same degree at fifteen to thirty minutes or two hours after injection of TSH (Fig. 7). In these experiments organic binding was permitted and the fraction of the glandular radioactivity present as inorganic iodide was comparatively small, the value in different experiments ranging between 0.32 and 0.59 per cent of the total, as determined electrophoretically. In certain experiments, I^{131} was given thirty minutes before right hemithyroidectomy; TSH was then given, and fifteen to thirty minutes later, left hemithyroidectomy was performed; in these cases TSH had little effect upon the proportion of glandular I^{131} present as iodide. In the TSH group (6 rats) there was a 29.1 per cent decrease in the proportion of glandular radioactivity present as iodide, and in the saline-

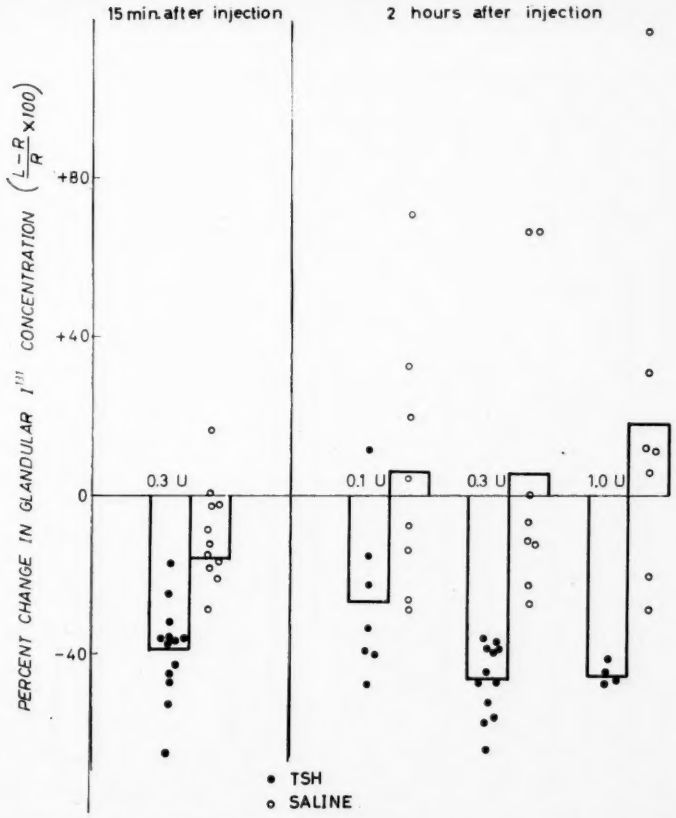

FIG. 6. Effect of TSH upon thyroidal iodide-I^{131} when organic binding was blocked. Rats were given propylthiouracil thirty minutes before administration of I^{131}. The right lobe was removed just before injection of the indicated dose of TSH or saline, and the left lobe removed fifteen minutes (left panel) or one hundred and twenty minutes (right panel) after the injection. The difference between the concentration of I^{131} in each lobe is expressed as a percentage of the concentration in the lobe removed first. Each point represents 1 animal; the height of the bars indicates the average value for each group.

injection controls (6 rats) there was a decrease of 34 per cent; the difference between the means lacked statistical significance.

DISCUSSION

These observations in man and in the rat tend to support the conclusion drawn from experiments in the dog that administration of TSH leads to the prompt release of inorganic iodide from the thyroid, the release pre-

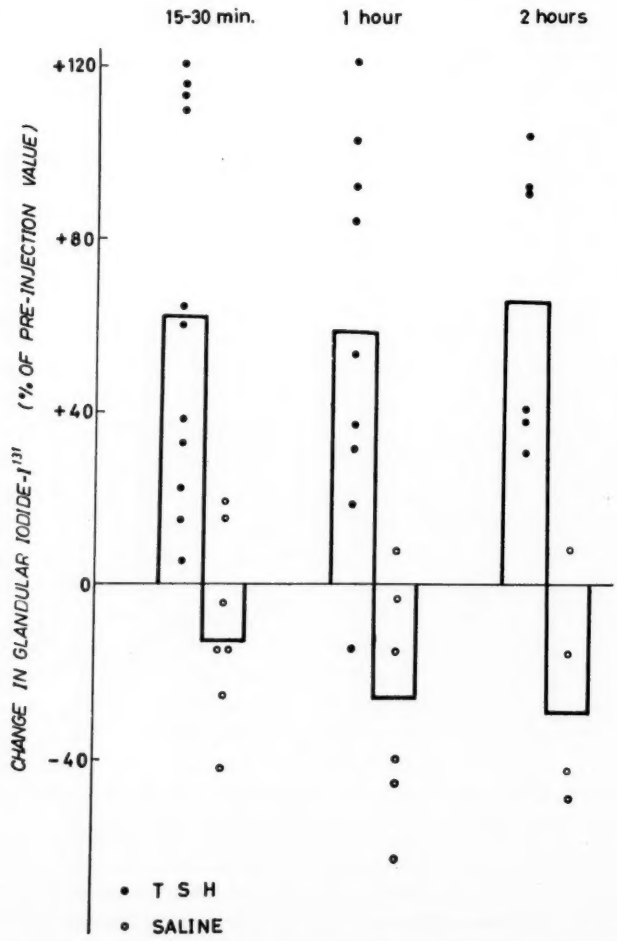

FIG. 7. Effect of TSH upon thyroidal iodide-I^{131}, when organic binding was not prevented. The right and left thyroid lobes of rats in which the thyroid glands had been labeled with I^{131} three days earlier, were removed, the interval between lobectomies being as indicated. TSH or saline was given intravenously immediately after removal of the right lobe. The difference between the fractions of the total I^{131} present as inorganic iodide in each lobe is expressed as a percentage of the fraction of iodide-I^{131} in the right lobe. Each point represents 1 rat. The height of the bars represents the average value for each group. $P<0.01$ for the difference between TSH and saline groups.

sumably being secondary to an increase in the iodide content of the gland (1, 3).

The urinary excretion of I^{131}, in rats and human subjects whose glandular iodine stores had been labeled, increased within a few hours after injection of TSH. These observations confirm those of Becker and co-workers (9), who studied euthyroid and hyperthyroid persons and concluded that administration of TSH led to a relative increase in the serum concentration of an iodine-containing substance which was rapidly cleared by the kidney. On the basis of the results described here, it would appear that the substance is inorganic iodide and that its source is the thyroid, from which it is rapidly released following the administration of TSH. That the enhanced renal excretion of I^{131} is not predominantly an extrathyroidal response to TSH is indicated by its absence in myxedematous patients and by the minimal changes in thyroidectomized rats. It is also unlikely that labeled iodide arising from the peripheral degradation of the increased quantity of thyroid hormones secreted in response to injection of TSH was a major source of the observed prompt increment in urinary I^{131}; the peak urinary excretion of I^{131} occurred within twenty-four hours after injection of TSH, whereas the maximal plasma concentration of PBI^{131} may not be attained for forty-eight hours or more after a single intramuscular injection of TSH (9–11). Since the half-life of thyroxine in plasma is normally six to seven days (12), and this is not changed by administration of TSH (13), most of the iodide arising from degradation of thyroxine secreted as a consequence of TSH administration would presumably be available for renal excretion only after an interval of some days following the injection.

An increased proportion of the glandular I^{131} was present as inorganic iodide within fifteen to thirty minutes after giving TSH to rats in which the thyroidal iodine had been labeled. This was not due to increased glandular accumulation of circulating iodide-I^{131} because (*a*) in rats in which the glandular iodine stores were poorly labeled and the circulating iodide was of higher specific activity, TSH did not increase the glandular iodide-I^{131}, and (*b*) a latent period of at least eight hours has been noted (14, 15) between the injection of TSH in the rat and the increased glandular uptake of I^{131}. It seemed plausible that the increase in glandular inorganic iodide found after injection of TSH arose from deiodination of organic compounds within the gland.

A period of fifteen to thirty minutes was required for the rise in the inorganic iodide content in the unblocked gland. Within the same interval of time, when organic binding was blocked, administration of TSH produced a prompt decrease in thyroidal $iodide^{131}$ content in the rat. In the experiments in which the interval between removal of the thyroid lobes

was fifteen minutes, there were probably no pronounced changes in plasma I^{131} concentration between the two operations, and the response may therefore be considered indicative of a reduction in the thyroid/plasma (T/P) iodide^{131} concentration ratio. In man, there was a similar response to TSH in most studies in which both thiocyanate and methimazole were given.

These observations could be explained on the basis of the prompt occurrence, after injection of TSH, of an increase in the glandular content of inorganic iodide (arising largely by deiodination), at least part of the iodide being released from the gland—particularly when the T/P iodide gradient was abolished by perchlorate, thereby leading to the increased renal excretion of iodide. When organic binding was blocked and glandular iodine stores were unlabeled, the administration of I^{131} permitted the thyroid to accumulate labeled iodide from the circulation. When TSH was given, stable iodide produced by deiodination of organic compounds could have mixed with the labeled iodide in the gland. The thyroid/plasma equilibrium might then be disturbed and a consequent accelerated release of labeled iodide might occur, *i.e.*, some of the trapped iodide might be "washed out" of the gland by the increment of iodide derived from deiodination. If a fixed quantity of unlabeled iodide were to enter the thyroidal iodide compartment and mix there with the labeled iodide which had been trapped from the circulation and was in equilibrium with the plasma iodide, one would expect that there would be an inverse relation between the amount of iodide initially present in the "trap" and the fraction of the label which would be released from the gland in the course of restoring the pre-existing stable iodide gradient. It is possible that the more consistent decrease in the glandular content of labeled iodide induced by TSH in persons whose thyroidal iodide space had been diminished by administration of thiocyanate, as compared with subjects receiving methimazole alone, may be explained in this way.

Halmi and his associates observed that TSH produced a prompt decrease in the thyroid/serum radioiodide concentration ratio in both hypophysectomized and intact rats (14, 15). They rejected the idea that this might be caused by flooding of the thyroidal iodide compartment with stable iodide derived from iodotyrosines, because the same response to TSH was observed in animals maintained with antithyroid drugs plus triiodothyronine, in which glandular iodine stores were therefore presumably greatly depleted (15). The implication was that insufficient iodide could have arisen from deiodination of residual organic iodine compounds. Even the prolonged administration of goitrogens, however, may not result in the complete disappearance of organic iodine from the thyroid (16, 17); glandular iodine concentration values of 1 to 3 mg. per cent have been reported for rats maintained with a goitrogenic diet before and after

hypophysectomy (16, 18) or the administration of thyroxine (16, 19). Since the quantity of glandular iodine available for deiodination under these conditions might not be negligible, one might question the validity of rejecting, on this basis alone, the "wash-out" mechanism previously discussed.

Acknowledgment

The authors are indebted to Miss N. Grimmett and Mr. L. Anderson for technical assistance.

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JCEM

THYROID SUPPRESSIBILITY AFTER THERAPY FOR THYROTOXICOSIS*

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ABSTRACT

Seventy-six thyrotoxic patients were studied by means of thyroid suppression tests (using thyroxine or triiodothyronine) after treatment with radioactive iodine. They were grouped according to the various intervals after therapy. Significant suppression of thyroid function was demonstrated as early as six weeks after treatment with radioiodine. There was close correlation between the degree of suppression achieved and the final clinical status of the patient. Suppressibility, absent in the thyrotoxic state, returns when the patient is rendered euthyroid.

INTRODUCTION

IT IS well established that the thyroid glands of thyrotoxic patients cannot be suppressed by the administration of thyroid hormone (1, 2). Reversion to normal suppressibility was mentioned by Morgans *et al.* (3) as a guide to the efficacy of treatment, although only a few cases were reported. Werner (4) studied suppression with triiodothyronine in patients who were rendered euthyroid by treatment with radioactive iodine or surgery. In the majority of patients treated with I^{131}, the thyroid could not be suppressed up to five years later; following surgery, however, normal suppressibility usually was evident within one year.

Suppression tests after treatment may be of value in assessing the response of the patient. In this paper we have attempted to interpret the result of the thyroid suppression test after radioiodine therapy for thyrotoxicosis.

METHODS

In all patients, the initial clinical diagnosis of thyrotoxicosis was confirmed by a thyroidal radioiodine uptake value well within the hyperthyroid zone (5), or by a suppression test value also clearly within that zone (6, 7).

Received September 16, 1960.

* Work partly carried out in the Joan Bonamy Research Laboratory and supported by a generous grant from an anonymous donor.

† Supported by a grant from the Australian Atomic Energy Commission.

The suppression test was performed by administering either thyroxine (T_4) in a dosage of 0.4 mg. per day for three weeks, or triiodothyronine (T_3) in a dosage of 120–150 μg. per day for one week. The uptake rate (k_1) was determined by the method of Oddie *et al.* (8) before and during the administration of these compounds. The ratio

$$\frac{k_1 \text{ before } T_3 \text{ or } T_4}{k_1 \text{ during } T_3 \text{ or } T_4}$$

was determined, and the logarithm of this ratio (x) was evaluated in relation to the diagnostic significance of suppression values previously reported (6, 7).

To determine the significance of changes in the value of x after therapy, comparison was made between the mean value of x at various times after therapy and the expected value of x in untreated thyrotoxic patients. The expected value of x was determined from previous experience with thyroxine and triiodothyronine suppression tests (6, 7), correcting for the relative number of patients tested with thyroxine (with and without prior antithyroid medication) or triiodothyronine at various time intervals after I^{131} therapy.

A clinical appraisal was made of each patient at the time of the suppression test.

RESULTS

The results of suppression tests on 76 patients who were treated with I^{131} for thyrotoxicosis are shown in Table 1. The data are classified according to the time that had elapsed between I^{131} treatment and the suppression test. The value of x was determined for each subject; the mean value of x after treatment differed significantly at each time interval from the mean value of x expected in a corresponding group of untreated thyrotoxic patients (6, 7).

The individual values of x were also classified according to their relation to the diagnostic limits of the test (6, 7), as "clearly hyperthyroid," "doubtful" or "clearly euthyroid." Comparison was made between these values and the clinical findings, similarly divided. This comparison was not made during the first six to eight weeks after therapy, since the clinical response lags behind the change in uptake rate. Table 2 shows that the results of a suppression test are generally compatible with the results of clinical assessment.

In an additional small series of 11 patients studied after prolonged antithyroid therapy or thyroidectomy, suppression could be demonstrated in all except those who were clinically thyrotoxic.

DISCUSSION

Significant suppression of thyroid function by the administration of T_3 and T_4 can be demonstrated as early as six weeks after treatment of thyrotoxicosis with radioactive iodine. This is all the more significant in the early periods after therapy, as a certain number of these patients were still clinically thyrotoxic and would be expected to have suppression values in the thyrotoxic range, thus lowering the mean value of x.

TABLE 1. THYROID SUPPRESSIBILITY AFTER I^{131} THERAPY FOR THYROTOXICOSIS

Results of thyroid suppression tests	Time after I^{131} therapy (weeks)						Totals
	0–6	6–12	12–24	24–36	36–52	>52	
No. of suppressions in the clearly thyrotoxic range	6	9	12	3	2	—	32
No. in the doubtful range	4	5	9	5	1	—	24
No. in the clearly euthyroid range	5	6	7	6	3	10	37
Totals	15	20	28	14	6	10	93
No. of suppressions with use of T_3	12	17	15	4	3	2	52
No. of suppressions with use of T_4 (no prior medication)	2	2	8	8	1	8	30
No. of suppressions with use of T_4 (prior antithyroid medication)	1	1	5	2	2	—	11
Totals	15	20	28	14	6	10	93
Mean x	$0.138 \pm$	$0.126 \pm$	$0.128 \pm$	$0.207 \pm$	$0.191 \pm$	$0.652 \pm$	
Standard error of mean	0.064	0.028	0.032	0.082	0.026	0.079	
Expected mean x in thyrotoxicosis*	$0.001 \pm$	$0.002 \pm$	$-0.004 \pm$	$-0.012 \pm$	$-0.003 \pm$	$-0.002 \pm$	
Standard error	0.019	0.016	0.016	0.075	0.037	0.080	
Probability of obtaining by chance this difference between the mean x's	$P<0.05$	$P<0.001$	$P<0.001$	$0.01<P<0.05$	$0.001<P<0.01$	$P<0.001$	

* Determined from the mean values of x in untreated thyrotoxic patients (6, 7) corrected according to the relative number of patients tested at each time interval with T_4 (with or without prior medication) or T_3.

TABLE 2. ASSOCIATION BETWEEN THYROID SUPPRESSION AND CLINICAL DIAGNOSIS AFTER I^{131} THERAPY

Suppression test classification	Clinical classification (number of patients)			Totals
	Clearly euthyroid	Doubtful	Clearly hyperthyroid	
12–24 weeks after therapy				
Clearly euthyroid	7	—	—	7
Doubtful	4	3	2	9
Clearly hyperthyroid	3	4	5	12
Totals	14	7	7	28
24–36 weeks after therapy				
Clearly euthyroid	5	1	—	6
Doubtful	4	—	1	5
Clearly hyperthyroid	1	—	2	3
Totals	10	1	3	14
>33 weeks after therapy				
Clearly euthyroid	13	—	—	13
Doubtful	—	1	—	1
Clearly hyperthyroid	—	1	1	2
Totals	13	2	1	16

The correlation between the clinical manifestations and the degree of suppressibility was close. When the suppression value was in the clearly euthyroid range, then it was almost certain that the patient would be found to be euthyroid clinically. However, in 3 patients who were considered to be euthyroid clinically, the suppression test values were clearly in the thyrotoxic range. These patients have been followed; 2 became clinically thyrotoxic and required further treatment; 1 is still being observed.

In 5 patients the suppression values were clearly in the thyrotoxic range, but the clinical symptoms were in the doubtful category. Three of these patients became clearly hyperthyroid and required further treatment; 1 later was considered euthyroid, and 1 is still being followed.

Of the 15 patients in whom suppression values were in the doubtful range, 8 were clinically euthyroid (later confirmed); 4 were in the clinically doubtful category (1 later became clearly euthyroid and 1 clearly hyperthyroid); and 3 who were clinically thyrotoxic later became clearly so and required further treatment.

Thyroid suppressibility was demonstrable in all 10 patients who were clinically euthyroid after thyroidectomy or prolonged antithyroid therapy.

Suppressibility of thyroidal iodine uptake returns when the thyrotoxic patient is rendered euthyroid, even though other features of the disease

such as exophthalmos (9) and abnormal metabolism of thyroxine (10) and triiodothyronine (11) persist. This suggests that the resistance to suppression in thyrotoxicosis is directly related to the hyperfunctioning thyroid gland and is not related to other possible etiologic factors. Werner (12) reported failure to suppress the 24-hour thyroidal I^{131} uptake in patients who were euthyroid clinically but had early eye signs of Graves' disease. However, we have been able to demonstrate (unpublished data) normal suppressibility in patients who were euthyroid clinically but who had exophthalmos of recent onset. This discrepancy could be explained if Werner's patients were subclinically thyrotoxic.

The pathways by which thyroid suppression occurs normally are well documented (13), yet the exact reason why suppression should not be demonstrable in hyperthyroidism is not known. Suppressibility returns soon after hyperthyroidism is cured by methods which, to the best of our knowledge, affect only the thyroid gland and its production of hormone. This suggests that some factor within the thyroid gland accounts for the resistance to suppression in patients with thyrotoxicosis, and this factor is operative only while the patient remains hyperthyroid.

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Letters to the Editor

ABSORPTION AND EXCRETION OF PARENTERALLY ADMINISTERED HUMAN PITUITARY GONADOTROPIN

To the Editor:

Gemzell *et al.* (1) obtained a partially purified "follicle stimulating" preparation from human pituitaries by means of a method based on calcium oxide extraction followed by ammonium sulphate fractionation. The clinical effect of this material by itself or in combination with human chorionic gonadotropin (HCG) was tested by the same investigators on 7 amenorrheic women. It was found that the administration of "FSH" alone produced polycystic enlargement of the ovaries and a pronounced increase in urinary estrogen output, whereas FSH and HCG combined produced, in addition, ovulation and a secretory transformation of the endometrium in the majority of the patients treated.

We administered a similar preparation to 5 male patients with pituitary gonadotropin deficiency. The main emphasis of the investigation, however, was on the absorption and subsequent excretion of the administered gonadotropin.

PATIENTS AND METHODS

Of the 5 male patients studied, 3 (Nos. 1, 2 and 4) had idiopathic eunuchoidism, 1 was a pituitary dwarf (No. 3), and 1 had been hypophysectomized because of a pituitary tumor (No. 5). All patients were characterized by lack of endogenous pituitary gonadotropin secretion.

The material administered was prepared by Dr. Gemzell in Stockholm by the method previously referred to. Twenty-four hours before use, it was dissolved in physiologic saline at a concentration of 0.5 per cent and sterilized by means of ultraviolet irradiation. As a rule no clear solution was obtained, but rather a homogeneous suspension which could be easily injected intramuscularly.

In all 5 patients the human pituitary gonadotropin was administered daily on three consecutive days at 8:00 a.m., intramuscularly. Patients 1, 2 and 3 received a dose of 30 mg. daily; Patients 4 and 5 received a daily dose of 60 mg. In a three-day control period preceding commencement of treatment, the urinary excretion of 17-hydroxycorticosteroids (17-OH-CS), 17-ketosteroids (17-KS) and "total gonadotropic activity" was measured and the plasma gonadotropin level was also checked.

Urinary gonadotropin was extracted by a modified kaolin-acetone method (2), and plasma gonadotropin by the method of Apostolakis (3). The results of the gonadotropin assays are expressed in terms of the unofficial reference standard, HMG 20A, 1 milligram of which corresponds by definition to 1 HMG unit. The mouse uterus test, the rat ovary test and the mouse ovary test on HCG-treated animals (HCG augmentation test) were used as bioassay end-points in assessing the material administered; only the mouse uterus test was employed for assays of plasma and urine gonadotropic activity. During the three days of treatment, urinary and plasma gonadotropin levels were again estimated. Blood samples were obtained daily three hours following injection, and on the third day an additional sample was drawn one hour after the injection. The foregoing determinations were repeated in the post-treatment period.

RESULTS

Potency of administered HPG. The potency was determined by bioassay, both before and after the ultraviolet irradiation. Table 1 shows the results obtained. It would appear that the sterilization process caused a certain loss of activity.

Gonadotropin excretion in urine. Figure 1 shows the results obtained during the pre-treatment, treatment and post-treatment periods. It is noteworthy that in all subjects the amount of excreted gonadotropin increased from day to day during the treatment period and that substantial amounts of gonadotropin were excreted for at least three days after cessation of treatment. It would seem that a certain "depot" effect was present. In all 5 cases, during the entire period in which urinary gonadotropin values were elevated, the total gonadotropin detected in the urine amounted to 4–7 per cent of the amount injected. Taking into account the fact that the yield by the urinary extraction method is more than 50 per cent, this would mean that not more than 10–15 per cent of the original amount is excreted in the urine. This is substantially less than the percentage reported by other investigators (4) using a purified preparation of HCG.

Gonadotropin levels in plasma. In Patient 5, the plasma on the third day of treatment contained 3.0 HMG units per 100 ml. three hours following injection of the gonadotropin preparation. In all other cases, both in the control and in the treatment period, the gonadotropic activity of the plasma was below the threshold of sensitivity[1] of the method (3.0 HMG units per 100 ml.).

Previous investigations in postmenopausal women (5) have shown that, for endogenously produced pituitary gonadotropin, the mean renal clearance is 0.18 ml. per minute (range, 0.04–0.43 ml.). A simple calculation

[1] When the method for the quantitative estimation of pituitary gonadotropin in plasma was first described (3) it was stated that the threshold of sensitivity is 10 HMG units per 100 ml. plasma. However, following the use of a more sensitive strain of mice for the bioassay the present threshold has been reached.

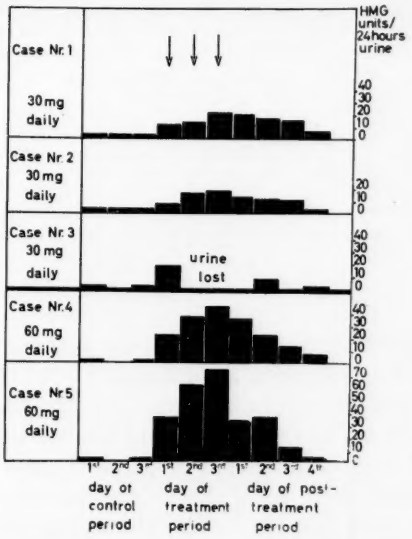

FIG. 1. Urinary excretion of gonadotropin following intramuscular administration of HPG.

shows that when the urinary excretion is more than 20 HMG units per twenty-four hours, gonadotropic activity is generally present in the plasma at levels exceding 3 HMG units per 100 ml.

Although urinary excretion of gonadotropin repeatedly surpassed 20

TABLE 1. GONADOTROPIC ACTIVITY OF THE ADMINISTERED PITUITARY EXTRACT IN HMG UNITS/MG. BEFORE AND AFTER ULTRAVIOLET IRRADIATION

	Bioassay	No. of animals	Gonadotropic activity (HMG units/mg.)
Before ultraviolet irradiation	Mouse uterus	16 16	48 24
	Rat ovary	16	29
	"HCG augmentation" test	16 16	32 21
		Mean ±S.E.*	31 ±4.8
After ultraviolet irradiation	Mouse uterus	16 16	22 27
	"HCG augmentation" test	16	20
		Mean ±S.E.	23 ±2.1

* Standard error.

TABLE 2. URINARY EXCRETION OF 17-KETOSTEROIDS AND TOTAL 17-HYDROXYCORTICOSTEROIDS BEFORE AND AFTER TREATMENT WITH HUMAN PITUITARY GONADOTROPIN

Patient No.	Dosage of pituitary gonadotropin	17-KS (mg./24 hrs.)		Total 17-OH-CS (mg./24 hrs.)	
		Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
1	3×30 mg.	9.3	11.6	5.7	6.7
2	3×30 mg.	19.9	21.8	8.1	8.3
3	3×30 mg.	2.3	11.6	6.0	11.2
4	3×60 mg.	17.1	12.9	12.5	14.5
5	3×60 mg.	6.7	10.9	5.1	5.0

units in the 5 subjects under discussion, no corresponding amounts of gonadotropin could be detected in the plasma. It would seem therefore that the administered material did not behave like the endogenous pituitary gonadotropin, at least in so far as the renal filter is concerned. The renal clearance of this extract was obviously higher than that of endogenous pituitary gonadotropin and, from the single observation in which both plasma and urine gonadotropin values were determined, the clearance approximated 1.7 ml. per minute. Although no final conclusion can be drawn, it is of interest that this value was about 8–9 times higher than that for endogenous pituitary gonadotropin and approximately double that for HCG (6). Whether this difference was due to a different molecular structure, to a possible linkage of endogenous gonadotropin to some other plasma protein which delays excretion, or to other unknown factors, is a matter for future elucidation.

Excretion of 17-KS and 17-OH-CS in urine. Table 2 shows the amounts of 17-KS and 17-OH-CS excreted in urine both before and after treatment with HPG. It is obvious that there was no significant effect. This is not surprising in view of the fact that the extract contained mainly follicle-stimulating activity and little interstitial-cell stimulating activity (1).

SUMMARY

Consecutively administered daily intramuscular injections of human pituitary gonadotropin extract serve to build up the concentration of gonadotropin within the body, an appreciable amount being present up to three days following the final injection.

Approximately 10–15 per cent of the total administered activity was excreted in the urine.

The relationship between plasma concentration and urinary excretion of the administered extract, as represented by the renal clearance, was different from that for endogenous pituitary gonadotropin.

The dosages used—up to 60 mg. (about 1400 HMG units) daily for three days—did not have any significant effect on the urinary excretion of 17-hydroxycorticosteroids and 17-ketosteroids.

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Acknowledgment

This work was supported by a research grant (WA 33) from the Population Council of the Rockefeller Institute.

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THE SUBLABIAL ABSORPTION OF SERUM GONADOTROPIN

Clinical acne vulgaris is said to be ameliorated by the sublabial administration of follicle-stimulating hormone (1–3). Although it is not known if the hormone is absorbed through the sublabial mucous membrane, the results of the following studies suggest that this is so.

METHODS

Rabbit antiserum was prepared by the method of Hayashida and Li (4), using an equine follicle-stimulating hormone preparation containing 2500 I.U. per mg. (Gonadyl Seric, Roussel) as antigen. The antigen-antibody reaction was detected by the precipitin

ring test (5) and a sensitized bentonite test (6) suitably modified. In the bentonite test a series of tenfold dilutions of serum gonadotropin were made with 0.9 per cent sodium chloride containing 1 per cent of normal male human serum. Two controls were used—a blank of diluent alone, and an antigen inhibition control prepared by adding 0.2 ml. of antiserum to 10 I.U. of serum gonadotropin thirty minutes before the test; 0.1 ml. of each dilution and of the controls was added to 0.4 ml. of physiologic saline in each of a series of tubes; 0.1 ml. of the sensitized bentonite suspension was added to each tube. The tubes were then shaken and examined for agglutination after standing for two hours at room temperature. Either of the tests could detect as little as 0.01 I.U. of gonadotropin added to 1 ml. of human serum.

One healthy human subject was given 5×50 I.U., and another such subject was given 2×50 I.U. of the hormone preparation sublabially. Blood was collected by venipuncture at appropriate times and the sera were tested for the presence of antigen.

RESULTS

The results are shown in Table 1. In Subject 1, sera separated from blood drawn after fifteen and thirty minutes, gave a weakly positive reaction corresponding to 0.01 I.U. of gonadotropin per ml. The sera of Subject 2 gave stronger reactions corresponding to 0.1 I.U. of gonadotropin per ml.

The antigenic material detected in the blood may not have been gonadotropin but an associated, biologically inactive antigen against which an antibody had been formed. However, in the precipitin ring test, a purer gonadotropin with a potency of 5350 I.U. per mg. also caused precipitation in a dilution containing 0.01 I.U. or more per ml. Moreover the test yielded positive results with horse serum against the rabbit antigonadotropin serum only in dilutions containing 0.05 mg. or more of protein per ml. The 0.01 I.U. of gonadotropin detectable in 1 ml. of serum after sublabial administration would be associated with about 4×10^{-6} mg. of the crude preparation, *i.e.*, much less than the amount of horse serum protein detectable. Serum gonadotropin diluted in aqueous solutions loses biologic activity within about eight hours at room temperature but not for some days at $-22°$ C. The immunologically detectable material in the serum after sublabial administration was similarly unstable (Table 2). Butt *et al.* (7)

TABLE 1. ANTIGEN-ANTIBODY TESTS ON SERUM FROM 2 SUBJECTS GIVEN SUBLABIAL SERUM GONADOTROPIN

Antigen-antibody test	Results in sera from blood samples drawn at			
	0 mins.	15 mins.	30 mins.	60 mins.
Subject 1				
Precipitin ring	negative	+	faint ring	negative
Sensitized bentonite	negative	+	+	negative
Subject 2				
Precipitin ring	negative	+	+	+
Sensitized bentonite	negative	+	++	++

TABLE 2. EFFECT OF STORAGE AT −22° C. ON ANTIGENIC ACTIVITY OF SERA FROM SUBJECT 2

Antigen-antibody test	Stored 24 hrs.			Stored 48 hrs.			Stored 72 hrs.		
	15-min. sample	30-min. sample	60-min. sample	15-min. sample	30-min. sample	60-min. sample	15-min. sample	30-min. sample	60-min. sample
Precipitin ring	+	+	+	negative	negative	negative	negative	negative	negative
Sensitized bentonite	+	+	+	negative	negative	+	negative	negative	negative

have shown that the antibody formed against a crude preparation of gonadotropin precipitates all the biologically active material from purified extracts of human menopausal gonadotropin.

The evidence therefore suggests that there is a parallelism between immunologic and biologic activity, and supports the hypothesis that serum gonadotropin can be absorbed sublabially in biologically active form.

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June 6, 1960

Acknowledgments

I am indebted to Miss L. Hopkinson and Miss M. Sandiford for their assistance in preparation of the antibody; to Professor C. H. Gray for his helpful advice and criticism; and to Dr. A. E. Gremeaux of Roussel Laboratories for kindly supplying the Gonadyl Seric tablets and the purified serum gonadotropin preparation.

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A DIURNAL PATTERN IN THE RATE OF DISAPPEARANCE OF I^{131}-LABELED *l*-THYROXINE FROM THE SERUM

To the Editor:

During our investigation of the effects of electroconvulsive therapy on the rate of disappearance from the serum of a single intravenous injection of *l*-thyroxine labeled with radioactive iodine (T_4-I^{131}), blood samples were drawn more frequently than usual in such a study. This modified technique revealed a "diurnal pattern" in the counts expressed as a percentage of the dose when plotted against time, as shown in a euthyroid subject in Figure 1. This letter is a preliminary report of the methods used and the results obtained in our investigation of this pattern in euthyroid patients.

CLINICAL MATERIAL AND METHODS

Patients

Eleven patients were investigated. Eight patients were euthyroid and without previous thyroid disease. Of these, 3 had schizophrenia, 1 had periodic catatonia, 2 had acute depressive psychosis, and 2 were free from mental disease. Three patients were euthyroid after therapy for thyroid disease; 2 with previous hyperthyroidism had been treated by thyroidectomy and by propylthiouracil respectively, and 1 with anterior pituitary insufficiency had been treated with desiccated thyroid, testosterone and cortisone.

All patients, regardless of their other illnesses, were euthyroid as judged clinically and by determination of serum protein-bound iodine (PBI) level and the rate of disappearance of T_4-I^{131} from the serum.

Methods

Serum PBI values were determined by a modification of the method of Barker *et al.* (1). For euthyroid adults our laboratory has established a range of 3.5–6.5 μg. per 100 ml. of serum.

The rate of disappearance of T_4-I^{131} was studied by the method of Sterling and Chodos (2, 3) and calculated from the values obtained on 8 a.m. serum samples by the method of least squares. In our experience the rate of disappearance in euthyroid subjects ranges from 10 to 14 per cent of the injected dose.

Twenty-four hours before the injection of the radioactive tracer, each patient was prepared with either 20 mg. of methimazole (Tapazole) or 200 mg. of propylthiouracil given every six hours to prevent recycling of I^{131} through the thyroid gland. This was continued throughout the study. The T_4-I^{131} (Abbott Laboratory, Oak Ridge, Tennessee) was diluted one hour before injection with a small amount of physiologic saline so that approximately 50 μc. would be present in 1 ml. of solution. A dose of 1 ml. of T_4-I^{131} was given intravenously at time zero.

Forty-eight hours after the tracer injection, when mixing and distribution were presumably uniform, a 10-ml. sample of venous blood was drawn from the opposite arm. These samples were obtained at approximately 8 a.m., 2 p.m., 8 p.m. and 2 a.m. daily for at least three days, the exact time of sampling being recorded. The blood was immediately refrigerated at 4° C. and the serum was separated two to thirty-six hours later by centrifugation; it was then stored until used in the study.

It has been shown in our laboratory that, for at least thirty-six hours, the length of

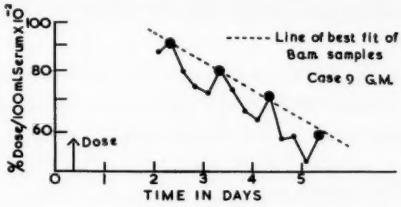

FIG. 1. Semilog plot of the diurnal patterns of disappearance of T_4-I^{131} from the serum in a euthyroid subject over a three-day period. The difference between the 2 a.m. and 8 a.m. sample amounted to approximately 13 per cent. However, when the differences presented by 11 patients and 37 days were considered, the mean difference was −8.4 per cent, as shown in Table 1. There was considerable variation among subjects and the range was −0.4 to −18.4 per cent for this difference.

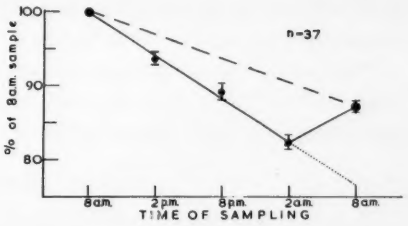

FIG. 2. Means and standard errors of the observed values for the disappearance of T_4-I^{131} from the serum over a 24-hour period (expressed as a percentage of the 8 a.m. values).

time that serum is in contact with the clot does not influence the serum thyroxine-I^{131} content.

When a study was completed, a 3.0-ml. aliquot of each of the foregoing samples was measured and counted in duplicate to ±2 per cent accuracy. To insure greater accuracy in measuring and counting, this aliquot was replaced in the original sample and the procedure repeated on another day. Counting was performed in a well-type scintillation counter (background 200 c.p.m.) against a standard made from an aliquot of the dose and expressed as a percentage of the dose per 100 ml. of serum. The total error of the method, as determined by 3 replicate estimations on the 45 serum samples used in 3 individual studies, ranged from 3 to 4 per cent.

Expression of results. The results were plotted semilogarithmically. Using only the 8 a.m. values within each study, a line of best fit by the method of least squares and a T_4-I^{131} disappearance rate[1] could be calculated for each patient. The deviation (in per cent) from the theoretical value on the line of best fit for each of the observed values was then obtained (Table 1). A positive difference represents an observed value above the line of best fit; a negative difference represents an observed value below this line. The percentage differences from the theoretical values on the line of best fit for each time of sampling were combined; for each group thus obtained the mean and standard error were calculated. P values were obtained from the "Student's" *t*-test tables.

All the observed values were expressed as percentages of the 8 a.m. value obtained at the beginning of each 24-hour period of observation. A mean and standard error for each time of sampling were calculated. The results for 37 samples are illustrated in Figure 2.

RESULTS

Thirty-seven diurnal patterns in 11 euthyroid patients were compared

[1] As programmed for the University of Toronto IBM computer by Dr. L. C. Lax, Research Fellow, Sunnybrook D.V.A. Hospital.

to the theoretical values on the line of best fit for all 8 a.m. samples. The results are summarized[2] in Table 1.

The 8 a.m. values did not deviate significantly from the calculated line of best fit. The 2 p.m., 8 p.m., and 2 a.m. values showed progressively greater deviation from this line; all of these differences were significant.

DISCUSSION

It is not the purpose of this preliminary communication to analyze the factors concerned in the rate of disappearance of T_4-I^{131} from the serum. The latter has been expressed in the literature as an exponential curve derived from single daily samples and T_4-I^{131} been shown to disappear from the

TABLE 1. DIURNAL PATTERN OF THE DISAPPEARANCE OF LABELED *l*-THYROXINE FROM THE SERUM IN 11 EUTHYROID SUBJECTS

Time of sampling	Means of % differences* (n = 37)	S.E.	P value (*t* test)
8 a.m.	+0.3	±0.54	.6
2 p.m.	−2.4	±0.81	< .01
8 p.m.	−4.1	±1.13	< .001
2 a.m.	−8.4	±0.93	< .001
8 a.m.	+0.2	±0.52	.7

* Per cent difference refers to the deviation of the observed value from the theoretical value on the line of best fit for all the 8 a.m. samples, expressed as a percentage of the theoretical value, as follows:

$$\frac{\text{observed value—theoretical value}}{\text{theoretical value}} \times 100.$$

serum of euthyroid subjects at a rate of approximately 12 per cent of the injected dose per twenty-four hours (2, 3). If T_4-I^{131} disappears from the serum at a constant rate, it would leave at a rate of approximately $\frac{1}{2}$ per cent per hour (dashed line, Fig. 2). Our data, as illustrated in Figure 2, indicate that in the first eighteen hours (8 a.m. to 2 a.m.) the rate of *disappearance* of T_4-I^{131} from the serum was approximately 1 per cent per hour, but that in the succeeding six hours the radioactivity *increased* approximately 1 per cent per hour to establish the average rate of 12 per cent for the total twenty-four hour period. Furthermore, if the earlier rate of disappearance of T_4-I^{131} from the serum were to continue from 2 a.m. to 8 a.m. (as illustrated by the dotted line in Fig. 2) it would result in the disappearance of 24 per cent of the injected dose by 8 a.m. rather than 12 per cent. To account for the discrepancy between the predicted and the observed values, the plasma radioactivity would have had to increase by 12 per cent of the injected dose during this time interval. This phenomenon may have been the result of changes in plasma volume.

Renbourn (4) observed a significant diurnal rhythm for hemoglobin,

[2] Detailed information about individual cases is available on request.

hematocrit and plasma protein levels. The changes reported for these values were as great as 10 per cent, or even more. The hemoglobin and hematocrit values fell progressively from 7 a.m. to 2:30 a.m. The plasma protein level did not change significantly from 7 a.m. to 10 p.m.; after 10 p.m. it fell significantly, and independently of bed rest. A slight rise was observed, beginning at 2:30 a.m.

In 2 groups (5, 6), including one from this center (5), no significant diurnal variation in the serum PBI level could be demonstrated. However, the error inherent in a serum PBI determination is high in comparison with that inherent in a serum protein determination. The standard deviation reported for duplicate determinations of the serum PBI level in the euthyroid range is ± 0.33 μg. per 100 ml. (7) compared with ± 0.05 Gm. per 100 ml. for determinations of the serum protein level (4). It is probable, therefore, that the PBI technique is not sensitive enough to detect the degree of biologic change involved in the observed diurnal variation in the rate of disappearance of T_4-I^{131}.

When 3 of the 11 patients reported here were given radioiodinated human serum albumin (RISA; Abbott Laboratory, Oak Ridge, Tennessee), using the same methods as for T_4-I^{131}, a similar diurnal pattern was noted (unpublished data). It would appear, therefore, that the diurnal pattern is not specific for T_4-I^{131} and is probably related to changes in plasma volume.

However, other possible contributing factors in the observed rise of serum radioactivity between 2 a.m. and 8 a.m. include a reduction in the rate of disappearance, or a return of radioactivity to the serum. The net contribution of these two factors may be assessed when serial blood volume changes can be measured concurrently with greater accuracy than is possible with the techniques presently available.

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PHILIP H. MELVILLE, M.D.†
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The Departments of Medicine, Pathological Chemistry and Psychiatry, University of Toronto; and the Clinical Investigation Units of the Toronto General Hospital, Toronto Psychiatric and Sunnybrook (DVA) Hospitals, Toronto, Ontario, Canada,
October 17, 1960

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Acknowledgments

Grateful acknowledgment is made to Professor W. H. Watson, Department of Physics and Director of the Computation Center, Dr. G. C. Gotlieb, Chief Computer, and Dr. L. C. Lax, for their help in the programming and calculating of our results; to Mrs. Denise de Pinto for her technical assistance; and to the nursing supervisors of the Clinical Investigation Units, Miss J. Oakes, Miss S. I. Rowland, and Mrs. D. Hammond, for their cooperation.

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ABNORMAL EXCRETION OF PREGNANETRIOLONE AND Δ^5-PREGNENETRIOL IN THE STEIN-LEVENTHAL SYNDROME*†

To the Editor:

Some patients with the Stein-Leventhal syndrome have adrenal hyperfunction, as manifested by elevated urinary androsterone, etiocholanolone and other individual 17-ketosteroids (1–3). Such increased production of urinary steroids, as well as the high incidence of hirsutism, indicates that there are abnormalities of steroid biosynthesis in this disease which may have biochemical features in common with the abnormalities noted in other disorders of adrenal steroidogenesis.

* Presented at the Forty-second Meeting of The Endocrine Society, Miami Beach, Florida, June 9–11, 1960.

† This work was supported in part by a grant from the New South Wales State Cancer Council, Sydney, Australia.

Recent studies of urinary steroids in congenital adrenal hyperplasia and in Cushing's syndrome with adrenal hyperplasia have shown that one of the results of the disturbances in adrenal steroid biosynthesis in these conditions is the appearance of pregnanetriolone in the urine; this steroid is not found in the urine of normal subjects (4, 5). In this preliminary study, the excretion of pregnanetriolone and two related steroids was determined in 6 Stein-Leventhal patients.

PATIENTS AND METHODS

Patients

Six women in whom the diagnosis of Stein-Leventhal syndrome was established, were studied. Bilateral ovarian wedge resection was performed in each case, and in all 6 there was histologic confirmation of the clinical diagnosis. The ages of the patients ranged from 21 to 36 years. Two of them were obese. Hirsutism was present in all 6, but only in one was it the primary complaint. All suffered from oligomenorrhea; the longest period of amenorrhea before operation was eighteen months. One patient (D.U.) gave a history of a full-term delivery thirteen years before the present study; this is unusual, but not unique. The other women were also married but were sterile. After bilateral ovarian wedge resection, all the patients menstruated regularly, but none showed any improvement in hirsutism. One became pregnant.

Urine samples were also obtained from 6 normal healthy women aged between 19 and 30 years. Estimations were carried out simultaneously with those for the Stein-Leventhal group.

Methods

The method of Cox (6) was used for the estimation of the acetaldehydogenic steroids, pregnanetriolone (pregnane-3α, 17α, 20α-triol-11-one), pregnanetriol (pregnane-3α, 17α, 20α-triol), and Δ^5-pregnenetriol (Δ^5-pregnene-3β, 17α, 20α-triol). Because of the low levels of pregnanetriolone expected, large aliquots of urine (250 ml.) were subjected to enzymatic hydrolysis. Extracts of each were chromatographed on strips (20 cm. wide) of Whatman No. 1 paper in ethylene-dichloride/formamide. Rechromatography of eluates for each steroid separately was carried out on paper lanes (1.5 cm. wide) in the benzene/formamide descending system, with overrunning sufficient to move each steroid a minimum of 25 cm. For accurate estimation and to check on the identity of the small amounts of pregnanetriolone noted, a further chromatogram in benzene/formamide as before was run with duplicate chromatograms containing added pure steroids, to compare the mobility of the urinary compounds and standards. Steroids were detected on the paper chromatograms, and semiquantitatively estimated by a phosphoric acid test. Final quantitative assay was by periodate oxidation and estimation of acetaldehyde.

RESULTS

The urinary excretion of pregnanetriolone, Δ^5-pregnenetriol and pregnanetriol by the Stein-Leventhal patients and the normal women is shown in Table 1. The results for the normal subjects were consistent with those reported previously (3, 5).

TABLE 1. ACETALDEHYDOGENIC STEROIDS IN THE URINE OF WOMEN WITH THE STEIN-LEVENTHAL SYNDROME AND OF NORMAL WOMEN

	Amount of steroid excreted		
	Pregnanetriolone (mg./24 hrs.)	Δ^5-Pregnenetriol (mg./24 hrs.)	Pregnanetriol (mg./24 hrs.)
Stein-Leventhal patients			
O.B., before op.	0.14	0.45	0.50
W.A., before op.	0.05	0.24	0.47
T.H., before op.	0.10	0.85	1.15
T.H., after op.*	0.04†		
H.Y., after op.	0.04†	0.29	0.41
M.A., after op.	0.05	0.35	0.30
D.U., after op.	0.10	0.30	1.12
Normal adult women			
G.H.S.	<0.01	0.11	0.60
J.M.	<0.01	0.06	0.33
T.W.	<0.01	0.13	0.91
M.S.	<0.01	0.08	0.73
E.C.	<0.01	0.12	1.01
M.T.	<0.01	0.21	1.41

* Patient 18 weeks pregnant, ten months after bilateral wedge resection.
† Estimation by phosphoric-acid fluorescence.

A main objective in the study was the investigation of the possible presence of pregnanetriolone in the urine of the Stein-Leventhal patients. Pregnanetriolone was found in the urine of each of the 6, in amounts ranging from 0.04 to 0.14 mg. per twenty-four hours. No pregnanetriolone was detected in the urine of the normal subjects, or at least it was less than 0.01 mg. per twenty-four hours. The identification of the measured compound as pregnanetriolone was based on: 1) paper chromatographic mobility in two solvent systems, with at least one high resolution run, 2) the characteristic blue-violet fluorescence obtained with pregnanetriolone in the phosphoric-acid test, 3) formation of acetaldehyde on periodate oxidation, a reaction specific for 17,20-dihydroxy-20-methyl steroids, and 4) a correlation of the results of 2) and 3).

Pregnanetriol excretion by the Stein-Leventhal patients was within the normal range, in agreement with the results of Lipsett and Riter (3). The Δ^5-pregnenetriol values in the Stein-Leventhal urines were unexpectedly high compared with those in the urine of normal subjects. For Patients T.H., O.B. and M.A., the Δ^5-pregnenetriol values were close to those for pregnanetriol, being 74, 90 and 117 per cent respectively of the pregnanetriol values. Although Δ^5-pregnenetriol appears to be present in urine as a 3β-sulphate, the procedure in use with hydrolysis by a limpet enzyme preparation is effective in liberating this steroid from its conjugate (7).

Both the preoperative and postoperative patients excreted pregnanetriolone and abnormally large amounts of Δ^5-pregnenetriol. The Δ^5-preg-

nenetriol excretion was lowest relative to pregnanetriol in Patient D.U., who had been operated on nineteen months previously.

DISCUSSION

The results show two previously undescribed alterations in urinary steroid metabolites in the Stein-Leventhal syndrome, *i.e.*, the appearance of pregnanetriolone, and an abnormally high excretion of Δ^5-pregnenetriol.

Pregnanetriolone is an abnormal urinary steroid; it has not been found in the urine of normal women, of pregnant women, or of men. In the extensive studies of Finkelstein (4) the average level of pregnanetriolone in normal urine was estimated to be less than 0.001 mg. per twenty-four hours. The conditions in which pregnanetriolone has been definitely identified in urine are congenital adrenal hyperplasia and Cushing's syndrome with adrenal hyperplasia. The values for pregnanetriolone in this group of Stein-Leventhal patients are somewhat lower than those reported for Cushing's syndrome with adrenal hyperplasia and much lower than those for congenital adrenal hyperplasia (4, 5). Recently Brooks *et al.* (8) found that pregnanetriolone was excreted in amounts of 1.0 mg. and 0.9 mg. per twenty-four hours by 2 subjects with the milder form of congenital adrenal hyperplasia manifested after puberty (8). Thus, the presence of pregnanetriolone in the urine from Stein-Leventhal patients suggests that the steroid abnormalities in this condition have some of the biochemical features of the abnormal steroid biosynthesis seen in adrenal hyperplasia.

The excretion of Δ^5-pregnenetriol by the Stein-Leventhal patients was higher than that for the normal subjects. However, the range of normal excretion of this steroid has not been well established and the Δ^5-pregnenetriol levels in the Stein-Leventhal patients shown in Table 1 may overlap the upper levels of the normal range for this steroid. As pregnanetriol excretion was within the normal range for this series of patients, it may be useful to compare the ratio of Δ^5-pregnenetriol to pregnanetriol rather than consider the absolute Δ^5-pregnenetriol values. The excretion of Δ^5-pregnenetriol is of interest in relation to previous observations of elevated androsterone and etiocholanolone excretion in Stein-Leventhal patients. A biosynthetic route to androgens, and thence to estrogens, is by way of 17-hydroxypregnenolone through dehydroepiandrosterone. Since 17-hydroxypregnenolone appears to be the precursor of Δ^5-pregnenetriol, a high urinary excretion of the latter may be correlated with high excretions of androsterone and etiocholanolone.

The source of the increased pregnanetriolone and Δ^5-pregnenetriol observed for the Stein-Leventhal patients is of interest. Alterations in steroidogenesis by the adrenals or the ovaries can be expected to result in changes in urinary steroid metabolites. The increase in 17-ketosteroids

observed previously is of adrenal origin (1). Pregnanetriolone is known only abnormally, and then as a metabolite of an adrenal steroid, whereas Δ^5-pregnenetriol is known to be increased in some adrenal disorders (5). Abnormal excretion of these steroids was observed in both preoperative and postoperative cases of Stein-Leventhal syndrome. However, the endocrine sources of the increased pregnanetriolone and Δ^5-pregnenetriol in this condition remain to be established.

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November 12, 1960

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The Endocrine Society

The Forty-Third Annual Meeting

General Information

Date: Thursday, Friday and Saturday,
June 22–23–24, 1961

Headquarters: Hotel Biltmore,
New York, N. Y.

Registration:

Fountain Room, Hotel Biltmore.
Everyone attending the meetings is required to register. By action of the Council, nonmembers will be charged a registration fee of $10.00 for any part of the scientific sessions.

Fellows, graduate students, residents and interns will be admitted upon presentation of proper credentials CERTIFIED BY DEPARTMENT HEAD OR SUPERINTENDENT OF HOSPITAL and payment of $2.00 registration fee.

Members should present their current membership cards when registering. Admission will be by badge only.

Your Endocrine Society badge will admit you to the American Diabetes Association's opening meeting Saturday morning at the Hotel Commodore. Members of the Endocrine Society are requested to register (without charge) at the desk of the American Diabetes Association.

Scientific Sessions:

All morning sessions will be held in the Ballroom. Simultaneous sessions will be held each afternoon in the Ballroom and the Bowman Room. Sessions will begin promptly on schedule. Presentation of all submitted papers will be LIMITED TO TEN MINUTES. All speakers will be required to adhere strictly to schedule. Speakers who have manuscripts for publication are invited to submit them to the Editor of ENDOCRINOLOGY or of THE JOURNAL OF CLINICAL ENDOCRINOLOGY AND METABOLISM.

Annual Dinner:

The Annual Dinner of the Society will be held on Friday evening, June 23, at 7:30 o'clock in the Ballroom. Cocktails will precede the dinner in the Fountain Room at 6:30 P.M. Secure tickets at time of registration.

Local Arrangements:

Dr. Nicholas P. Christy is Local Chairman and Mrs. Warren O. Nelson is in charge of the Women's Entertainment Committee.

Program

THURSDAY, JUNE 22, 1961

8:00 a.m.—Registration—Fountain Room, Hotel Biltmore

SESSION I. 9:00 a.m.—PITUITARY

Room: Ballroom

Co-Chairmen: *Roy O. Greep*
Alfred E. Wilhelmi

1. Chromatography of Canine Hypothalamic Extracts.
   Andrew V. Schally, Harry S. Lipscomb, John M. Long (by invitation), *Wayne E. Dear* (by invitation) and *Roger Guillemin.*
2. Evidence for Central Nervous System Inhibition of ACTH Secretion.
   Richard H. Egdahl.
3. Influence of Alterations in Structure of the N-Terminal Serine on Corticotropin Activity.
   Harold E. Lebovitz (by invitation), *Frank L. Engel, H. B. F. Dixon* (by invitation) and *Lowell R. Weitkamp* (by invitation).
4. Diffuse Hypothalamic System in the Regulation of ACTH Release.
   A. Brodish.
5. Effects of Reserpine and Hypothalamic Lesions on Pituitary Lactogen Content in the Rabbit.
   S. Kanematsu (by invitation), *J. Hilliard* (by invitation) and *C. M. Sawyer.*
6. Secretion of Prolactin by Pituitary *in Vitro.*
   Joseph Meites, Raymond H. Kahn and *C. S. Nicoll* (by invitation).

INTERMISSION

7. Biologic Activities of Human Hypophysial Mammotrophin.
   W. R. Lyons, Choh Hao Li and *Ruth E. Johnson* (by invitation).
8. Effect of Experimentally-Induced Hypo- and Hyperthyroidism upon Pituitary Lactogenic Hormone Concentration.
   Clark E. Grosvenor.
9. Immunochemical Studies on Human Growth Hormone in Blood and Urine.
   Selna L. Kaplan (by invitation) and *Melvin M. Grumbach.*
10. Further Evidence for Amino Acid Transport as a Site of Action of Growth Hormone.
    E. Knobil and *P. F. Brande* (by invitation).
11. Extraction of Vasopressin by Percolation.
    Murray Saffran, Brigitte U. Caplan (by invitation), *Seymour Mishkin* (by invitation) and *Brahm Muhlstock* (by invitation).
12. Three Types of Basophils of the Human Adenohypophysis and the Hormones They Produce.
    Calvin Ezrin and *William C. Nicholas.*

THURSDAY, JUNE 22

SESSION II. 2:00 p.m.—PITUITARY; REPRODUCTION (FEMALE)

Room: Ballroom

Co-Chairmen: *James T. Bradbury*
Melvin M. Grumbach

13. Specific Hypothalamic Sites at Which Progesterone Acts to Facilitate Ovulation in the Rat.
Charles A. Barraclough and *S. Yrarrazeval* (by invitation).
14. Sexual Differences in the 24-Hour Rhythm of Pituitary-Adrenal Function in Rats.
Vaughn Critchlow, Harry S. Lipscomb, Robert A. Liebelt (by invitation), *Mildred Elwers* (by invitation) and *Walter E. Mountcastle* (by invitation).
15. A New Parameter for Biological Characterization of Gonadotropins: Species Differences and Significance for Bioassay.
Albert F. Parlow.
16. Changes in Pituitary LH Content During the Rat Estrous Cycle.
Neena B. Schwartz and *Delphine Bartosik* (by invitation).
17. The Time Required for Release of Ovulation-Inducing Hormone from the Rat Hypophysis After Stimulation of the Preoptic Hypothalamus.
John W. Everett and *H. M. Radford* (by invitation).
18. A Hypothalamic Luteinizing Hormone (LH)-Releasing Factor.
S. M. McCann.

INTERMISSION

19. Effect of Exogenous Human FSH on Ovaries and Ovarian Steroids.
Robert B. Greenblatt and *Virendra B. Mahesh* (by invitation).
20. An Electron Microscope Study of the Ovarian Response to Gonadotrophin.
James A. Green (by invitation).
21. Gonadal Disorders with Unusual Chromosomal and Karyologic Findings.
Avery A. Sandberg, George F. Koepf, and *Thomas F. Frawley.*
22. Importance of Oxidation Reactions in the Initiation of Uterine Growth by Estradiol.
E. V. Jensen, J. W. Flesher (by invitation), *N. N. Saha* (by invitation) and *H. I. Jacobson* (by invitation).
23. Radioautographic Studies of the Decidual Reaction in Pseudo-Pregnant Mice Treated with Tritiated-Thymidine.
Key June Seoung (by invitation).
24. Enzymes in Rat Uteri During Post-Partum Involution.
Bertha L. Lobel (by invitation) and *Helen Wendler Deane.*

THURSDAY, JUNE 22

SESSION III. 2:00 p.m.—ADRENAL

Room: Bowman Room

Co-Chairmen: *William W. Tullner*
Don H. Nelson

25. Fractionation of Crude Kidney Extracts for Aldosterone Stimulating Activity.
James O. Davis, Elwood Titus (by invitation), *Herbert E. Spiegel* (by invitation), *Carlos R. Ayers* (by invitation), and *Charles C. J. Carpenter* (by invitation).
26. Effect of Angiotensin and ACTH on Adrenocortical Secretion in Hypophysectomized Nephrectomized Dogs.
William F. Ganong, Patrick J. Mulrow and *Gerald Cera* (by invitation).
27. Aldosterone Biosynthesis: The Influence of Various Precursors, ACTH and Angiotensin II.
Norman M. Kaplan, and *Frederic C. Bartter.*
28. Recent Observations on Adrenoglomerulotropin.
Gordon Farrell.
29. Synthesis of Aldosterone in Hypertensive Congenital Adrenal Hyperplasia with "Complete" Deficiency of 11β-Hydroxylation.
Donald A. Holub and *Joseph J. Barlow* (by invitation).
30. The Turnover Rate, Distribution, and Plasma Binding of Aldosterone in Pregnancy.
D. S. Layne (by invitation), *B. Little* (by invitation), *C. J. Meyer* (by invitation), *J. F. Tait* and *S. A. S. Tait.*

INTERMISSION

31. Studies of Adrenocortical Function of Hirsute Women.
Ross D. Jacobs, Julia Lobotsky (by invitation), *Arnold M. Moses, Edward L. Klaiber* (by invitation) and *Charles W. Lloyd.*
32. Impaired Steroid Biosynthesis in Adrenocortical Cancer.
Mortimer B. Lipsett and *Hildegard Wilson.*
33. Renal Unresponsiveness to Salt Retaining Hormones in Infancy.
G. Leboeuf (by invitation), *D. D. Steiker* (by invitation), *A. M. Bongiovanni* and *W. R. Eberlein.*
34. Adrenal Inhibitors and Stimulators.
Joseph J. Barlow (by invitation) and *Donald A. Holub.*
35. Reduction of Adrenal Steroid Production by an Inhibitor of Cholesterol Biosynthesis (MER-29).
James C. Melby, Sidney L. Dale (by invitation) and *Marlys St. Cyr* (by invitation).
36. Two New Inhibitors of the Adrenal Cortex.
J. J. Chart and *H. Sheppard* (by invitation).

FRIDAY JUNE 23

8:30 a.m.—Registration, Fountain Room

SESSION IV. 9:00 a.m.—REPRODUCTION (MALE; FEMALE)

Room: Ballroom

Co-Chairmen: *E. Perry McCullagh*
Sheldon S. Segal

37. Dichotomous Effect of Bis-(Dichloroacetyl) Diamines upon Spermatogenesis, Leydig-Cell and Gonadotropin Function in Man.
Carl G. Heller, Donald J. Moore (by invitation), *Yves Clermont* (by invitation) and *C. Alvin Paulsen.*

38. Urine Estrogen Response to Human Chorionic Gonadotrophin (H.C.G.) in Young and Hypogonad Men.
W. I. Morse, Albert Clark (by invitation), *W. A. Ernst* (by invitation) and *C. L. Gosse* (by invitation).
39. Steroidogenesis in the Human Foetal Testes.
Hernan F. Acevedo, Leonard R. Axelrod, Eisel Ishikawa (by invitation) and *F. Takaki* (by invitation).
40. Estimation of the Secretory Rate of the Testicular Precursors of Urinary 17-Ketosteroids.
Paul C. MacDonald (by invitation), *Raymond L. VandeWiele* and *Seymour Lieberman.*
41. The Effect of 19-Hydroxyandrostenedione on the Aromatization of Δ^4-Androstenedione-3,17.
Nina Hollander (by invitation).
42. Further Studies in the Renal Handling of Endogenous Estrogens in Late Pregnancy.
C. H. Brown (by invitation) and *John R. K. Preedy.*

INTERMISSION

43. The Use of Bromine-82 in the Analysis of Estrogens.
W. R. Slaunwhite, Jr. and *L. Neely* (by invitation).
44. Steroidogenesis in the Polycystic Ovary.
Leonard R. Axelrod and *Joseph W. Goldzieher.*
45. Regulation of Nucleic Acid Synthesis by Androgens.
Charles D. Kochakian and *David G. Harrison* (by invitation).
46. A New Pathway for the Biosynthesis of Testosterone.
Enrico Forchielli (by invitation), *Marcel Gut* (by invitation) and *Ralph I. Dorfman.*
47. Stimulation of Incorporation of Acetate into Testosterone by FSH and LH *in Vitro.*
Peter F. Hall (by invitation) and *Kristen B. Eik-Nes.*
48. Hormonal Factors in the Adrenal-Dependent Growth of the Rat Ventral Prostate.
William W. Tullner.

FRIDAY, JUNE 23

1:30 p.m.—ANNUAL BUSINESS MEETING

Room: Ballroom

SESSION V. 2:30 p.m.—THYROID

Room: Ballroom

Co-Chairmen: *Joseph Edward Rall*
Samuel P. Asper

49. Detection of Antibodies to Thyroglobulin by Paper Electrophoresis of Serum Containing Thyroglobulin-I^{131}
I. N. Rosenberg and *F. Pamukcu* (by invitation).

50. Immunologic Properties of Thyrotropin.
Richard P. Levy, William L. McGuire (by invitation) and *Lawrence Heideman, Jr.*
51. Transplacental Transfer of Thyroid Isoantibodies in the Rabbit.
William H. Beierwaltes, Katherine E. Elzinga (by invitation), *Robert W. Schmidt* (by invitation), *Vernon N. Dodson* (by invitation), *Norma R. Spafford* (by invitation) and *Edward A. Carr, Jr.*
52. Correlation of Thyroid Hormone Metabolism and Effect in *Necturus maculosus*, A Thyroxine-Resistant Species.
Valerie Anne Galton and *Sidney H. Ingbar.*
53. An Evaluation of the Physiological Role of Prealbumin in the Binding of Thyroxine.
C. S. Hollander (by invitation), *V. V. Odak* (by invitation), *T. E. Prout* and *S. P. Asper, Jr.*
54. Thyroxine-Binding in Human Fetal Serum.
M. Andreoli (by invitation) and *J. Robbins.*

INTERMISSION

55. Normal PBI and Decreased Serum Thyroxine-Binding in Two Sisters with Hyperthyroidism.
R. R. Cavalieri (by invitation).
56. The Interrelationships Between Fatty Acids and TSH Action in Sheep Thyroid Tissue.
Norbert Freinkel and *Edward C. Saef* (by invitation).
57. Effect of Hypophysectomy and TSH on Synthesis and Secretion of Thyroglobulin Precursor in Thyroid Epithelium.
N. J. Nadler and *B. Mitmaker* (by invitation).
58. Urinary Catechol Amines and VMA in Patients with Hyperthyroidism and Hypothyroidism.
John G. Wiswell, Gilbert E. Hurwitz (by invitation), *Victor Coronho* (by invitation) and *Oscar H. L. Bing* (by invitation).
59. Stimulation of Glucose-6-C^{14} Metabolism in Rat Thyroid Slices by Propylthiouracil (10^{-8}M).
Philip F. Mulvey, Jr. (by invitation) and *D. Ward Slingerland.*
60. Inhibition of Thyroidal I^{131}-Utilization *in Vitro* by an Intrathyroidal Substance.
Winton Tong (by invitation) and *I. L. Chaikoff.*

FRIDAY, JUNE 23

SESSION VI. 2:30 p.m.—CARBOHYDRATE, LIPID, AND CALCIUM METABOLISM

Room: Bowman Room

Co-Chairmen: *Leslie L. Bennett*
Francis D. W. Lukens

61. Direct Effect of Insulin on Liver Slices from Depancreatized Frogs.
J. C. Penhos (by invitation).

62. Contribution of the Pentose Cycle to Glucose Metabolism in Rat Adipose Tissue *in Vitro*.
Bernard R. Landau and *Jack R. Leonards* (by invitation).
63. Effect of Mannoheptulose on Blood Glucose and Ketone Bodies in the Fasting Rat.
Robert O. Scow, Ernst Simon (by invitation) and *Sidney S. Chernick* (by invitation).
64. Effect of Hypophysectomy on Metabolic Changes Produced by Growth Hormone in Juvenile Diabetes.
Dorothy H. Henneman, Eleanor McGarry and *John C. Beck*.
65. A Simplified Technique for Measurement of Insulin Binding Globulin
G. J. Dendrinos (by invitation), *T. E. Prout* and *V. V. Odak* (by invitation).
66. Insulin Resistant Diabetes Associated with Increased Endogenous Plasma Insulin Followed by Remission.
James B. Field.

INTERMISSION

67. An Insulin Secretion and Storage Defect in Certain Insulinomas.
R. H. Pollen (by invitation), *G. M. Grodsky* (by invitation) and *V. C. DiRaimondo*.
68. Effects of Human Growth Hormone on Lipid Metabolism in Diabetic Subjects.
R. E. Visintine (by invitation), *G. D. Michaels* (by invitation) and *L. W. Kinsell*.
69. The Specific Structure of Compounds Effecting Fat Mobilization in Man.
Morton D. Bogdonoff, Joseph W. Linhart (by invitation), *Robert F. Klein* (by invitation) and *E. Harvey Estes, Jr.* (by invitation).
70. Effects of Vitamin D and Cortisone on Intestinal Absorption of Calcium in the Rat.
Gerald A. Williams, E. Nelson Bowser and *Walter J. Henderson* (by invitation).
71. Use of Continuous Infusions of Calcium-45 to Study Skeletal Metabolic Function.
Clayton Rich.
72. Balance Studies of Calcium Loading in Osteoporosis.
Ernest Schwartz (by invitation), *William V. Chokas* (by invitation) and *Richard L. Fogel* (by invitation).

FRIDAY, JUNE 23

SESSION VII. ANNUAL DINNER—Hotel Biltmore

John E. Howard, President, presiding

6:30 p.m. Cocktails—Fountain Room.
Sponsored by the donors of the Awards of the Society: Ciba, Ayerst, Upjohn and Squibb.
Attendance limited to holders of dinner tickets.

7:30 p.m. Dinner—Ballroom.
Presentation of the 1961 Awards of the Society by *Dr. E. Perry McCullagh*
The Fred Conrad Koch Award
The Ciba Award
Ayerst Fellowship
Upjohn Scholar

President's address: *Dr. Howard*

SATURDAY, JUNE 24

8:30 a.m.—Registration, Fountain Room

SESSION VIII. 9:00 a.m.—ADRENAL (continued)

Room: Ballroom

Co-Chairmen: *Frederic C. Bartter*
Ralph I. Dorfman

73. 6-β-Hydroxy-Cortisol: Origin in Normal Subjects and Elevation by Estrogen Treatment.
Marvin M. Lipman (by invitation), *Fred H. Katz* (by invitation), *Andrew G. Frantz* (by invitation) and *Joseph W. Jailer.*

74. Isolation and Properties of the Corticosteroid Binding Globulin (Transcortin) from Normal Human Plasma.
Ulysses S. Seal (by invitation) and *Richard P. Doe.*

75. Study of 17-Hydroxyprogesterone-4-C^{14} in Man.
David K. Fukushima, H. Leon Bradlow, Leon Hellman, Barnett Zumoff (by invitation) and *T. F. Gallagher.*

76. Rate of Production and Degradation of Cortisol in Obesity. *J. P. Eckert* (by invitation), *O. C. Green* (by invitation) and *C. J. Migeon.*

77. Suppression of Pituitary ACTH Response in the Human by ACTH Administration.
John E. Plager and *Paul Cushman, Jr.* (by invitation).

78. Studies on the 21-Hydroxylase and Δ^5-3β-Hydroxysteroid Dehydrogenase of the Human Adrenal.
Lorna J. Langer and *Steven M. Podos* (by invitation).

INTERMISSION

79. Hormonal Effects on Distribution of Amino Acid Radioactivity in the Adrenal of the Male Rat.
E. D. Bransome, Jr. (by invitation) and *W. J. Reddy.*

80. Glucose Metabolism in the Adrenal Gland.
Vernon K. Vance (by invitation), *Francois Girard* (by invitation) and *George F. Cahill, Jr.*

81. Biosynthetic Capabilities of Isolated Adrenal Reticuloendothelial Cells.
David L. Berliner, C. J. Nabors, Jr. (by invitation) and *Thomas F. Dougherty.*

82. Isolation of 18- Hydroxycorticosterone from Rat Adrenals.
Fernand G. Peron.

83. Effect of Saluretic Agents on Blood Pressure and Thyroid Activity of Hypertensive Rats.
Melvin J. Fregly.

84. ACTH Induced Changes in the State of Oxidation of Adrenal Ascorbic Acid.
Boyd W. Harding (by invitation), *Elmer R. Rutherford* (by invitation) and *Don H. Nelson.*

SATURDAY, JUNE 24

SESSION IX. 2:00 p.m.—SYMPOSIUM ON PARA-ENDOCRINE DISORDERS

Room: Ballroom

Chairman: *John E. Howard*

2:00–2:20: Derangement of Calcium Metabolism in Sarcoidosis (see abstract 89).
Norman H. Bell, John R. Gill, and *Frederic C. Bartter,* National Heart Institute, Bethesda, Md.

DISCUSSION

2:30–2:50: Malignant Tumors Mimicking Hyperparathyroidism (see abstract 100).
Thomas B. Connor, William C. Thomas, William B. Stodghill and *John E. Howard,* The Johns Hopkins University and The University of Maryland School of Medicine, Baltimore, Md.

DISCUSSION

3:00–3:30: Severe Cushing's Syndrome Apparently Due to Corticotropin-Secreting Oat Cell Carcinoma (see Abstract 162).
Clifton Meador, Donald Island, Wendell Nicholson, John Nuckton, Charles Lucas, John Luetscher and *Grant Liddle,* Departments of Medicine, Vanderbilt University and Stanford University, Nashville, Tennessee and Stanford, California.

DISCUSSION

3:30–3:50: Polycythemia Secondary to Neoplasms (no abstract).
Thomas A. Waldmann, National Cancer Institute, Bethesda, Md. (by invitation).

DISCUSSION

4:00–4:20: Disturbances in Carbohydrate Metabolism in Cancer Patients (no abstract).
Paul A. Marks, Columbia University College of Physicians and Surgeons, New York City (by invitation).

The 1961 Awards

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by the Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors. Endocrine Society awards are limited to citizens of the United States and Canada.

Fred Conrad Koch Award

In 1957 a substantial legacy was bequeathed to the Society by the late Elizabeth Koch for the purpose of establishing the Fred Conrad Koch Memorial Fund in memory of her late husband, Distinguished Service

Professor of Physiological Chemistry at the University of Chicago and pioneer in the isolation of the androgens. This is the highest honor of The Endocrine Society and is represented by a medal known as the Koch Medal of The Endocrine Society. The medal, as well as an honorarium of $3,500, is to be given annually to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada.

This Award was first presented in 1959, to Dr. W. W. Swingle and in 1960 was presented to Dr. Emil Witschi. The Koch Medal has replaced the Medal of The Endocrine Society which was established in 1954.

The Ciba Award

The Ciba Award is given in recognition of the meritorious accomplishments of an investigator in the field of basic or clinical endocrinology who has not reached his thirty-sixth birthday before June 1 of the year in which the Award is presented. This Award was established in 1942 and first presented in 1944. The Award is presently in the amount of $2,500.

The Ayerst and the Squibb Fellowships

The Ayerst Fellowship was established in 1947 and the Squibb Fellowship in 1956. They are designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. Each Fellowship is awarded on alternate years and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

The Upjohn Scholar of the Endocrine Society

The Council of The Endocrine Society has established a category of Scholars. A grant has been made available through the generosity of the Upjohn Company, and will be awarded to an established investigator or teacher in the field of endocrinology who wishes to extend the opportunities for work either in this country or abroad.

The award will not exceed $2,500 annually and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Fred Conrad Koch Award, the Ciba Award, and the Ayerst and the Squibb Fellowships may be made by any member of The Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *October 15* each year.

Proposals for appointments as a Scholar of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *October 15* each year.

Thirteenth Postgraduate Assembly in Endocrinology and Metabolism

Under the Co-Sponsorship of The Endocrine Society
and The National Institutes of Health
Bethesda, Maryland
October 2–6, 1961

A comprehensive review of clinical endocrine problems and current research activity in these areas will be presented. For further information, write to: Dr. Roy Hertz, National Institutes of Health, Building 10, Bethesda 14, Maryland. The fee will be $100.00 for physicians, with a reduction to $30.00 for Residents and Fellows. Enrollment limited to 100.

Course in Clinical Radioisotope Instrumentation—Oak Ridge Institute of Nuclear Studies

A five-day course entitled "Advances in Clinical Radioisotope Instrumentation" will be offered *October 23–27, 1961*, at the Medical Division of the Oak Ridge Institute of Nuclear Studies, Oak Ridge, Tennessee.

The course is designed for physicians and paramedical personnel who already have experience in handling radioisotopes. Limited to forty participants, the course will consist of lectures and demonstrations and will cover theory and practical aspects of liquid, crystal, and gas detectors. The sessions will include topics on circuitry, electronics, data processing, scanning, dosimetry, and calibration.

Application forms and additional information are available from Ralph M. Kniseley, M.D., Medical Division, Oak Ridge Institute of Nuclear Studies, P. O. Box 117, Oak Ridge, Tennessee.

Fifth Pan American Congress of Endocrinology

The Fifth Pan American Congress of Endocrinology will meet at the Faculty of Medicine in Lima, Peru, *October 15–21, 1961*. The lecturers and participants in round table discussions will be: Drs. A. Albert, R. Caldeyro-Barcia, W. Buño, C. H. Li, J. Beck, A. Atria, R. I. Dorfman, L. Soffer, G. Thorn, D. Ingle, I. Bonomo, A. Hurtado, G. Farrell, F. Croxatto, J. A. García Reyes, J. Tait, R. Iglesias, W. Neuman, P. Talalay, R. Hall, M. Roche, E. del Castillo, A. Alpert, R. Noble, G. Pincus, A. Zaffaroni, R. Greenblatt, L. Wilkins, J. Conn, S. Zubirán, F. de la Balze, L. Gardner, M. San Martin, W. Nelson, J. Stanbury and F. Elmadjian.

The program sessions are in the following order: Pituitary Hormones, Adrenal Cortex, Hormones and Tumors, Nature of Hormone Action, Thyroid Function, Hormones and Reproduction, Pancreas and Diabetes, and Hormones and Behavior.

Short communications will also be accepted *until July 15, 1961*. All information regarding the meeting, hotel accommodations, and tours can be obtained from: Dr. Mauricio San Martin, P. O. Box 1161, Lima, Peru.